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## THE TRANSMISSION OF IMPULSES THROUGH A SYMPATHETIC GANGLION

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In 1907 Elliott pointed out a number of striking resemblances between characteristics of the motor plates of skeletal muscle and the cell bodies in sympathetic ganglia. Three of them are pertinent here. 1. Nicotine in small concentration stimulates typical activity in ganglion cells and induces twitches in muscle fibers; in greater concentration it paralyzes both the cells and the fibers (Langley, 1901, 1907). 2. Curare paralyzes the "motor nerve endings" of skeletal muscles; larger doses paralyze also autonomic ganglion cells (Langley and Anderson, 1895). And 3, preganglionic fibers can make functional union with skeletal muscles, and *vice versa*—an interchange not possible with the ordinary postganglionic sympathetic fibers (Langley and Anderson, 1904).

These similarities at synaptic junctions in striate muscle and in sympathetic ganglia have been extended by recent evidence that the process of transmission is alike in the two regions. Thus, 4, acetylcholine is produced in a sympathetic ganglion when preganglionic fibers are stimulated (Feldberg and Gaddum, 1934; Barsoum, Gaddum and Khayyal, 1934), and in skeletal muscle when motor nerves alone are in action (Dale and Feldberg, 1934; Dale, Feldberg and Vogt, 1935). 5. Acetylcholine delivered in minute amount *via* the blood vessels to these junctions stimulates ganglion cells to discharge impulses (Feldberg and Vartiainen, 1934) and also evokes muscular contractions, e.g., of the gastrocnemius (Simonart and Simonart, 1935; Brown, Dale and Feldberg, 1936). Furthermore, 6, when acetylcholine is injected so that it is present in excess it, like nicotine, has a completely paralyzant effect on ganglion cells (Feldberg and Vartiainen, 1934) as well as on motor plates of skeletal muscle (Rosenblueth, Lindsley and Morison, 1936). The evidence that the functional conditions at synapses in ganglia and in end plates of striate muscle are alike in these six respects

presents the question whether there may not be other similarities, possibly significant of events occurring in the two regions.

We shall call attention first to observations and explanations of phenomena which attend stimulation of skeletal muscle through its nerve at different rates and in different circumstances (Rosenblueth and Morison, 1937). 1. When a sufficiently rapidly repeated tetanizing current is applied persistently to a motor nerve the muscle contracts, then relaxes more or less, then enters into a further contraction which is followed by a slow relaxation as the current continues. This plus-minus-plus-minus sequence can be explained by assuming, on the basis of facts enumerated above, that the quickly recurring nerve impulses at first discharge acetylcholine which evokes contraction and then produce it in such excess that the primary stimulant effect is shifted to a depressant action (inducing the relaxation); that with persistence of these impulses the output of acetylcholine becomes gradually less until the concentration drops from the depressant to the stimulant range and the secondary contraction therefore occurs; and that the final failure to maintain the original height of the tetanus results from a further decrease of acetylcholine until it is below threshold for some cells—a state which may be regarded as due to “fatigue.” 2. As a protector of acetylcholine from destruction by an esterase, eserine or its effective equivalent, prostigmin, when injected at the start of a tetanic stimulation, hastens the primary relaxation because it favors an increase of the depressant concentration of the mediator; during the last fatigue period, when the output of acetylcholine is diminished, an injection of prostigmin, by preserving some of the amount produced, causes an increased contraction. If a muscle is stimulated through its nerve by a series of spaced single shocks, the resultant twitches become larger after eserine because the acetylcholine liberated by each shock is kept from prompt destruction and induces repetitive stimulation of the muscle (Brown, Dale and Feldberg, 1936). 3. Curare, by raising the threshold of excitation against acetylcholine (Briscoe, 1936), may have two opposite effects: if given at that stage of tetanic stimulation when acetylcholine is present in excess and when, consequently, the response is reduced or abolished (e.g., after eserine), contraction occurs; on the other hand, if given at the subsequent, fatigue stage, curare produces relaxation. These phenomena were illustrated and the explanations offered were discussed by Rosenblueth and Morison in their study of the effects of different rates of stimulation and different drugs on muscular performance induced by nervous impulses in conditions of natural blood supply. On the basis of the analogy between sympathetic ganglia and motor plates of skeletal muscle would similar phenomena appear at ganglionic synapses, *with natural circulation*, when preganglionic fibers are stimulated and the same drugs are employed? That was the question which prompted the present research.

**METHODS.** For examination we selected the superior cervical ganglion (s.c.g.) of the cat. It is easily approached, the preganglionic (pre-g.) fibers are readily isolated, there is a sufficient length of postganglionic (post-g.) fibers to permit electrodes to be placed upon them, and the nictitating membrane (n.m.) affords a graphic indicator of activity, whether started in pre- or post-g. axons.

The cats were under dial anesthesia (0.7 to 0.8 cc. per kgm.). A cannula was fastened in the trachea. The right femoral vein was bared for injections. Whenever acetylcholine (a-ch.) was to be used the adrenal glands were tied off in order to prevent a confusing discharge of adrenaline. For stimulation of the ganglion the cervical sympathetic strand was isolated from the vagus low in the neck, it was crushed caudad, and the shielded electrodes applied to it were padded with dry absorbent cotton and covered with the skin. If the post-g. fibers were to be stimulated as a control, the vagus cephalad of the jugular ganglion and also the glossopharyngeal nerve in the same region were excised, and the fibers cephalad of the s.c.g. were carefully isolated, crushed at their emergence from the ganglion, and gently lifted onto shielded electrodes, further protected by absorbent cotton and skin.

The n.m. was fastened to a recording lever, magnifying about 10-fold. If the movements of both membranes were registered simultaneously the cat's head (in a Czermak clamp) was held horizontally; otherwise it was tilted to favor the single membrane being used.

For the tetanizing current the ordinary Harvard inductorium was employed, with 6 volts in the primary circuit. It delivered about 90 shocks (make and break) per second. The secondary was usually placed at 8 or 9 cm. The single shocks were discharges from condensers having capacities between 0.005 and 2  $\mu$ F.

In order to prevent the unfavorable effects of eserine on the organism atropine (1 mgm. per kgm.) was first injected, intravenously. In our experience atropine, if given during continuous contraction of the two n.ms (one stimulated through pre-g., the other through post-g. fibers, with a tetanizing frequency), causes temporarily a lower contraction of the membrane receiving impulses through the s.c.g. and has no noteworthy effect on the other membrane, i.e., its effect is depressant on the ganglionic functions. Although eserine was occasionally employed, the more stable prostigmin (Roche), which may properly be substituted for eserine (physostigmine; cf. Easson and Stedman, 1936), was the chief reliance in protecting acetylcholine from rapid destruction. It is worthy of mention that even prostigmin has a demonstrable action for only a brief time—15 to 20 minutes (see fig. 5)—and that later doses are likely to be more effective than the first.

In a few of the first experiments a small dose of cocaine was used to sensitize the response of the n.m.

**RESULTS.** In the ganglion the range of concentration of a-ch. between too little to serve as a stimulus and an excess which depresses can readily be demonstrated. If within a short period after an injection of prostigmin (3 mgm.) increasing amounts of a-ch. are given, there is on the ganglion side (g. n.m.), but not on the other side (non-g. n.m.), a sudden large change from a threshold to a nearly maximal response with only a small change of dose (e.g., from 0.25 to 0.5 mgm.); and when the dose is made larger (e.g., 2 mgm.), the maximal efficiency may be passed (see fig. 1). Thus 5 mgm. may reduce the response to nearly half that evoked by 1 mgm. It is noteworthy that the most effective dose of a-ch. caused no marked contraction of the non-g. n.m. either directly, or indirectly by ex-

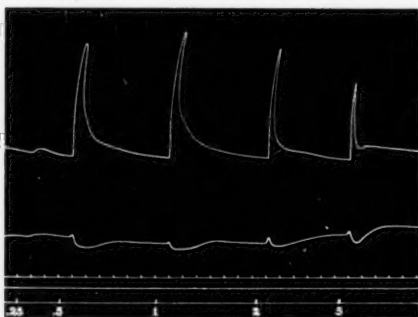


Fig. 1

Fig. 1. Effects of increasing doses of a-ch. on responses of n.m. Upper record, s.e.g. present; lower, absent. Doses (intravenous) in milligrams. Time intervals in this and other records, half-minutes. (Dial, atropine, adrenals out.)

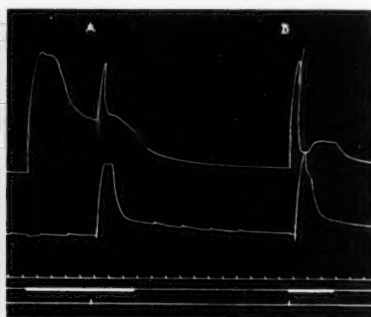


Fig. 2

Fig. 2. Effects of a-ch. at different phases of tetanic stimulation of pre-g. fibers. Upper record, tetanic, and lower record, single shocks, applied to pre-g. fibers. At signal-marks a-ch. (1 mgm.) injected intravenously.

citing sympathetic ganglia to discharge sympathin; possibly the belated rise after the dose of 5 mgm. may have been due to this indirect action (Bacq, 1936).

*The effects of single and rapidly repeated shocks on the s.e.g.* As indicated by contraction of the n.m., maximal single shocks, delivered to pre-g. or post-g. fibers every half-minute or every minute, have uniform effects (see fig. 3).

To a tetanizing stimulation of the pre-g. fibers, the n.m., unlike skeletal muscle, does not normally respond by a plus-minus-plus-minus oscillation of the height of contraction (see p 222), but by a plus-minus sequence (see figs. 2, 6 and 8). If the four phases of skeletal muscle are numbered 1-2-3-4, it appears that the response of the ganglion is from 1 to 4 (as later evi-

dence will indicate), with omission of 2 and 3. In skeletal muscle the effects, 1-2-3-4, were obtained only when the nerve was stimulated at nearly maximal frequency—240 shocks per second. Stimulation of the ganglion through the pre-g. fibers at the rate of 120 or 180 shocks per second elicited no other form of contraction of the n.m. than that elicited when the rate was 90 per second—the rate of the induction coil. Absence of the temporarily depressed phase in the response (phase 2) might be attributed first to a remarkable efficiency in the destruction of a-ch. If that were the sole factor concerned, however, it should have its limit at which a-ch., not destroyed, would accumulate and block transmission. As noted, this phase does not appear. Again, it might be supposed that the rate at which the pre-g. fibers may carry impulses would be so low as to render rapid stimulation futile; then complete destruction of the a-ch. might be achieved between relatively infrequent impulses, in spite of rapid stimulation. But a number of observers have found that the absolutely refractory period of these fibers is about 2 msec.—a figure which Brown (1934) has given also for the ganglion and the post-g. fibers. With the usual relation between the absolutely and the relatively refractory periods, the cervical sympathetic should be able to carry to the n.m. about 100 full-sized impulses per second. Brown's data just mentioned were obtained by giving one or two pre-g. shocks. The question arises as to whether these data really disclose conditions which prevail when the shocks are rapidly repeated. Is transmission in the ganglion, when it is subjected to repetitive stimulation, the same as it is in the nerve fiber? There is good evidence that it is not. Rosenblueth, Davis and Rempel (1936) found that continued shocks at 100 per second on post-g. fibers evoked a well-sustained response of the n.m., but that continued shocks more frequent than about 20 per second on the pre-g. fibers resulted soon in failure to maintain the initial contracted state. Obviously some condition in the ganglion limited the efficacy of the rapidly recurrent stimuli—90 per second—which we employed.

With no evidence of an accumulation of a-ch. either as a depressant block or as an occasion for an after-discharge, there remains, besides the possibility of swift destruction, a second possibility, especially when rapidly repeated shocks are used—that of a quick fall in the rate of production of a-ch., down to a level where the esterase present would be quite able to cope with it. In that condition no depressant excess would develop; and therefore no phase 3 would appear, because there would be no occasion for a drop of an excess to a point where stimuli are again effective. The phases would shift directly from 1 to 4.

On the second suggestion, offered above, there is recent information. In the s.c.g. perfused with Locke's solution Brown and Feldberg (1936) found that even when the stimulation rate on the pre-g. fibers was not greater

than 17 shocks per second there was a sudden high outburst of a-ch. from the ganglion, followed by a rapid fall to about one-fifth the original amount. With a normal blood supply conditions were, of course, not the same in the ganglion we studied, but the strikingly different effects of pre-g. and post-g. stimulation—the decreasing contraction in the former, the maintained contraction in the latter (see fig. 8, and cf. Orias, 1932)—can best be explained by assuming that in the ganglion the tetanizing current (90 shocks per second) soon evokes an output of a-ch. below the threshold for some of the cells—i.e., there is a state of fatigue. That rapid repetition of the stimuli plays an important rôle in producing this state is indicated by Orias' observations that with a selected low frequency of stimulation of

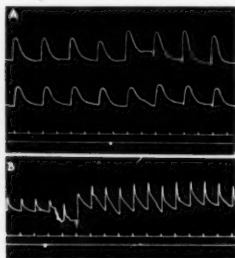


Fig. 3

Fig. 3A. Effects of prostigmin (after cocaine) on responses of n.m. to single maximal shocks. Upper record, pre-g. stimulation; lower, post-g. At signal-mark prostigmin (0.5 mgm.) injected intravenously (Dial, atropine, adrenals out, cocaine (2 mgm. per kgm.)), right s.e.g. (lower record) crushed.

B. Potentiation of responses of n.m. by prostigmin (without cocaine). Single maximal shocks applied to pre-g. fibers. At signal-mark, prostigmin (5 mgm.) injected intravenously.

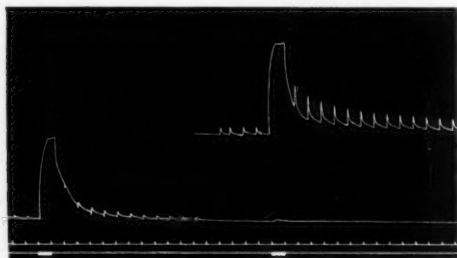


Fig. 4

Fig. 4. Effect of tetanic stimulation on the responses to single maximal shocks, at half-minute intervals. Upper record, electrodes on pre-g. fibers; lower, on post-g. fibers. (Dial, adrenals out, no drugs.)

pre-g. fibers the n.m. may be kept maximally contracted for at least an hour, and that after the fatigue state has been produced by rapid stimulation, lowering the frequency causes the contraction to become greater. Evidence as to whether there may be an approach to excessive production of a-ch. near the start of repetitive stimulation, as Brown and Feldberg's perfusion experiments suggest, and also evidence as to whether the smaller height of contraction, which follows the primary maximum in the response of the n.m. to a pre-g. tetanizing current, is the consequence of excess or deficiency of a-ch. can be tested by giving a-ch. or prostigmin.

*The effects of prostigmin.* This drug has been tried in three ways: by local application to the s.e.g., by injection of a small amount (1 $\gamma$ ) into the

common carotid artery (with external and internal branches tied), and by injection into the femoral vein. The last method was found most satisfactory.

As indicated by the n.m. prostigmin has no direct peripheral influence nor does it influence the magnitude of the contractions elicited by stimulation of the post-g. fibers (see fig. 3). It definitely prolongs the action of a dose of a-ch. that causes a contraction of the n.m.

On the ganglion prostigmin may have various effects. By itself it may induce a slow contraction and relaxation of the n.m. If a series of periodic single shocks is in progress, the contractions then rise on the elevated base line, and at the peak the cycles of contraction may be slightly prolonged and the heights of contraction increased (fig. 3). Occasionally the result is initially depressant on the ganglion, and, as recovery takes place, the individual contractions and relaxations are longer than before. A third influence of prostigmin is that of producing an almost pure potentiation (see fig. 3B). This observation confirms the observations of Feldberg and Vartiainen (1934), and is not in accord with Eccles' (1934) negative testimony. These various effects are reported merely as phenomena which have been noted. The reason for their variety is not evident, and probably would require considerable experimentation before being discovered (cf. Feldberg and Vartiainen, 1934). The directly significant feature of the action of prostigmin is revealed when a tetanizing current is used.

When prostigmin is given shortly before a series of brief tetanic stimulations of pre-g. fibers, the results are as those shown in figure 5. At first the height of contraction is reduced because, while the stimulus is being applied, the contractile process suddenly ceases and is replaced by relaxation. At the beginning of the next stimulation, a few minutes later, the contractile process is again interrupted, but the n.m. relaxes only to a moderate degree when it is driven into contraction—a true plus-minus-plus response. In the later stimulations the relaxations become much less prominent and the contractions rise to approximately the original height.

On the basis of earlier considerations, especially as related to the events in the neuro-muscular junction of skeletal muscle, the phenomena recorded in figure 5 can be explained, as follows. The first contraction (B) after prostigmin was not of normal height because the first outburst of a-ch. in the ganglion—then the greatest output, according to Brown and Feldberg (1936)—is protected against prompt destruction and therefore accumulates to a depressant concentration; the ganglion is momentarily paralyzed, it ceases to transmit the pre-g. impulses and the n.m. therefore relaxes. The subsequent recovery, demonstrated by the series (C, D and E), occurs because prostigmin has a temporary influence; if the gradual disappearance of the relaxation were due to ganglion fatigue the final contractions would become gradually less instead of gradually greater. As the protective

service of prostigmin is reduced the a-ch. is destroyed at more-and-more nearly the normal speed until at last only a slight remnant of the initial excess is present, as indicated by a brief check in the contractile phase.

The paper by Brown and Feldberg (1936), on the output of a-ch. from the s.c.g., was published while the experiments here reported were in progress. These investigators likewise obtained a plus-minus-plus response from the n.m., but only when the s.c.g. was perfused with an eserized Locke solution and shocks were delivered to the pre-g. fibers at the rate of 11 per second. Their explanation of the first minus phase, like ours, is that it results from excess of a-ch. in the ganglion: conditions which lessen the output of a-ch. make it disappear, conditions which increase the presence of a-ch. accentuate the depression, and if the a-ch. in the ganglion is not protected by eserine the inhibitory effects are not seen. The failure of

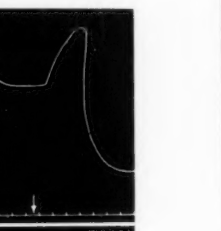
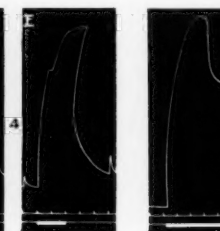
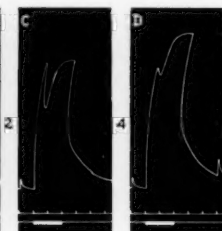
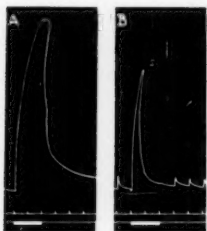


Fig. 5

Fig. 5. Effects of prostigmin on recurrent maximal tetanic stimulation of pre-g. fibers: A, response of the n.m. after atropine (4 mgm.) but before prostigmin; B, 3 minutes after prostigmin (2 mgm.); intervals between records indicated in minutes. (Secondary coil at 8 cm.)

Fig. 6. Effect of prostigmin on the late stage of tetanic stimulation of pre-g. fibers. At signal-mark prostigmin (1 mgm.) injected intravenously.

Brown and Feldberg to disclose the phenomenon in the ganglion normally supplied with blood is explained, we suggest, by the relatively slow rate of stimulation which they employed. It permitted the a-ch. to be destroyed fast enough to prevent a depressant accumulation.

The rise in the height of the contraction during the last two stimulations (D and E) in figure 5 can reasonably be regarded as the natural consequence of a destruction of a-ch. at the rate of its output (so that it does not accumulate) and a consequence also of the maximal output in the early stage of repetitive stimulation.

Although near the start of stimulation prostigmin may lead to the interrupted contractile process shown in figure 5, it later has quite the opposite effect. In figure 6 is shown what occurs when a small amount of prostigmin is injected during the period which follows the initial plus phase

of a tetanic stimulation of pre-g. fibers. Earlier it was suggested that prostigmin could be used to determine whether the lower contraction of this period is due to excess or deficiency of a-ch. If due to excess the protective prostigmin would augment the excess and by paralyzing the ganglion would lower still more the contraction; if due to deficiency it might raise the contraction by preserving a-ch. and increasing its concentration until it rises above threshold for the previously idle cells. The latter possibility alone has received experimental support. A dose of prostigmin which, when given before tetanic stimulation is started, induces sharp relaxation, causes, when given at the later period, a marked rise followed by a fall, sometimes below the starting level (see fig. 6). This evidence supports the view expressed above that normally the ganglion cells, in a possible plus-minus-plus-minus sequence attending rapidly repeated stimuli, pass directly from the first to the last phase.

*Effect of acetylcholine.* As might be expected, the influence of a-ch. on the s.e.g. when it is being stimulated rapidly (90 shocks per second, on the pre-g. fibers) depends upon the phase existent when the substance is injected. When the ganglion is nearly exhausted, as manifest by low contraction of the n.m., a small dose of a-ch. (1 mgm.) may have a purely stimulant effect, which is not so great nor so persistent as it is on the non-fatigued side (see fig. 2). At an intermediary phase of partial fatigue the same dose may evoke a momentary rise in the level of contraction, followed by a quick fall and gradual recovery (see fig. 7). And when injected at the start of a brief tetanic stimulation it causes a sharp drop of the contraction which has started, an effect just opposite to that evoked on the non-stimulated side (see simultaneous ordinates, fig. 2B).

The pure rise on the tetanized side in figure 2A is explicable as the consequence of sufficient addition of a-ch. to that being produced by the many fatigued nerve terminals, to bring enough idle cells into action to mask any fall which might occur because of a depressant effect on cells already discharging. The plus-minus-plus effect of a-ch. shown in figure 7 would be the natural result of a primary addition to a subthreshold concentration, restoring cells to activity, and, very quickly, the presence of an excess of a-ch. which partially paralyzes the ganglion; as the excess disappears the pre-g. impulses liberate approximately the same quantity of a-ch. as before the injection, and the contraction of the n.m. returns to approximately its former level. When the a-ch. was injected at the start of stimulation it summed with the initial burst of a-ch. occurring at that time (see fig. 2), and thereby was produced, on the stimulated but not on the non-stimulated side, a depressant excess.

The point should be clear that the effects of a-ch. are wholly consistent with and fully support the argument based on the results from prostigmin; at the start of stimulation both are depressant (a consequence explicable

as due to excess of a-ch.), and later, during the phase of low contraction of the tetanically stimulated n.m., both are stimulant (a consequence explicable as due to deficiency of a-ch. in the ganglion).

*Effect of curare.* As previously mentioned, curare, in its influence on skeletal muscle, acts as if it raised the threshold of stimulation against a-ch. On the assumption that it would act similarly on the s.c.g., it might be expected to exert different effects in accordance with the quantity of a-ch. present. If a-ch. should be present in excess, curare might increase the discharge from the ganglion and raise the height of contraction of the n.m., because it would lessen the efficacy of the depressant concentration.

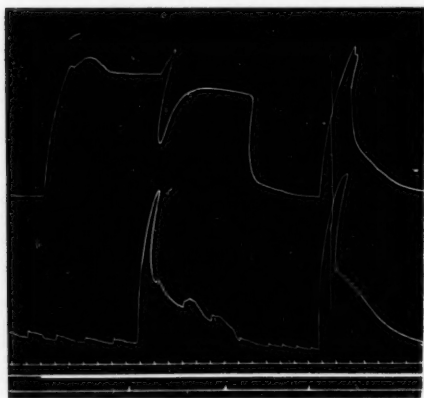


Fig. 7

Fig. 7. Effects of a-ch. before and after curare when pre-g. fibers are stimulated tetanically (upper record) and with single shocks at minutes or half-minutes (lower record). At first and last signal-marks, a-ch. (1 mgm.) injected intravenously; at the middle mark, curare (0.2 cc.).

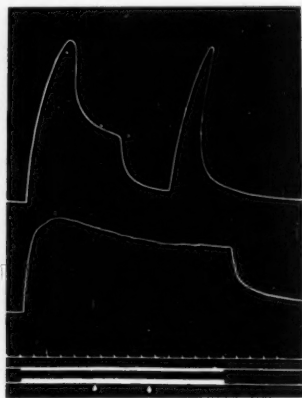


Fig. 8

Fig. 8. Effect of prostigmin after curare when pre-g. fibers (upper record) and post-g. fibers (lower) are stimulated tetanically. At first signal-mark intravenous injection of curare (0.2 cc.); at second mark, prostigmin (2 mgm.).

On the other hand, if there should be a deficiency of a-ch., curare might lower the height of contraction by increasing the number of subthreshold units.

The effect of curare in the fatigued condition is readily demonstrated (see fig. 7). The injection of a small dose (slightly more than the amount just necessary to produce complete paralysis of skeletal muscles) causes a quick paralysis of the ganglion when it is in phase 4.

To show the influence of curare in releasing the cells of the s.c.g. from the depressant effect of an excess of a-ch. is more difficult. Twice, after producing the relaxation which appears when stimulation follows prostig-

min (see fig. 5B), we have brought about a slight contraction by injecting curare. The effective dose in these instances was relatively small. Since time-consuming adjustments of the dose of curare to a period of evanescent excess of a-ch. in the ganglion would be required to obtain further evidence on this point, and since the point did not seem especially significant, we have not labored it.

The obverse of the release of ganglion cells from a depressant excess of a-ch. by giving curare—i.e., the release of the cells from the depressant influence of curare by giving a-ch.—is easily shown. Figure 7 illustrates a prompt contraction of the n.m. when, after curare, there is added to the amount of a-ch. produced by tetanic stimulation of the pre-g. fibers a small intravenous injection. The a-ch. injected was sufficient to cause a discharge of the other ganglion as well, but the importance of the stimulated output was proved by the efficacy of smaller doses of a-ch. (0.1 and 0.2 mgm.) on the stimulated side, as contrasted with a lack of any effect on the side not stimulated.

The protective service of prostigmin, also, can permit an accumulation of a-ch. in the ganglion that will overcome the depressant effect of curare and make the n.m. contract temporarily (see fig. 8).

That these antagonistic processes occur in the ganglion was shown by absence of noteworthy alteration of the record if post-g. fibers were stimulated, while a simultaneous record of the effects when pre-g. fibers were stimulated displayed the changes above described (see fig. 8).

*Effect of tetanic stimulation of the s.c.g. on the efficacy of single shocks.* A phenomenon characteristic of skeletal muscle, when stimulated through its nerve, is a post-tetanic increase of the response to single shocks of uniform maximal intensity (Schiff, 1858). Other resemblances between the reactions of a sympathetic ganglion and the motor plate raise the question as to whether the similarity can be extended also to this phenomenon. As shown in figure 4, brief application of a tetanizing current to the pre-g. fibers results in a remarkable change—single shocks become much more effective after the tetanic stimulation than they were before. The explanation of this change is not clear. Probably it is due to the liberation of some adjuvant agent at the transfer point. As figure 4 demonstrates, there is, after the tetanus, a slight increase in the height of contractions when the maximal single shocks are delivered to the post-g. fibers. This might result because each shock adds sympathin to a residuum of sympathin temporarily left from the tetanus. We have noticed that when a small dose of adrenaline (30  $\gamma$ ) is injected during a series of maximal shocks of post-g. fibers at 30-second intervals, the contractions of the n.m., shortly after the injection, may be more than twice as great as they were before it—an instance of cooperation of sympathin and adrenaline (cf. Rosenblueth and Cannon, 1932).

For the post-tetanic increment which results from pre-g. stimulation the addition of sympathin to residual sympathin in the n.m. may play a rôle, but the effect is of such different order of magnitude, as compared with post-g. stimulation, that changes in the ganglion must be considered. The effect lasts too long to be a potentiation due to a-ch. residual after the tetanus. It might be a potentiation from liberated potassium (cf. Feldberg and Vartiainen, 1934). In any case it must be an indirect action through post-g. fibers. Only two explanations of an increased height of contraction in these circumstances are known—spatial and temporal summation. In these experiments spatial summation is excluded because a maximal stimulus, when delivered to pre-g. fibers, has the same effect as when delivered to post-g. fibers (Brown, 1934), i.e., impulses in all the pre-g. units induce a discharge of all post-g. units. Spatial summation ruled out, only temporal summation remains. We may conclude, therefore, that each maximal shock applied to pre-g. fibers, that previously evoked a single discharge from the ganglion cells, was effective after the tetanus in evoking repetitive discharges. We have attempted to corroborate this conclusion by recording the action potentials of post-g. fibers and of the n.m. Although changes were registered in the records after a brief tetanus, they were not sufficiently clear to permit a discrimination between repetitive discharges and temporal dispersion.

**DISCUSSION.** The sympathetic ganglion differs from the motor plate of skeletal muscle in relations, structure and apparent functions. And yet, as the figures and descriptions in the foregoing pages reveal, their responses to arriving nerve impulses and to the action of drugs are remarkably alike. The stimulant effect of a small dose of a-ch., the depressant effect of a large dose; the primary depression of the tetanic response after prostigmin, the rise in the response when prostigmin is given late during a tetanus; the reduction or abolition of the synaptic transfer of impulses by curare, the restoration of the transfer by prostigmin; the decurarizing influence of a-ch., and the post-tetanic increase of the efficacy of single shocks—all these phenomena, similar in neuromuscular and ganglionic synapses, point definitely to the occurrence of processes which are qualitatively similar, if not identical, in the two regions.

In criticism of the theory of chemical transmission of nerve impulses the argument has been advanced that the evidence for an output of a-ch. at the synapses now being considered has been derived solely from experiments in which the structures were perfused with salt solution. That argument is not pertinent to the results here reported. Our results were obtained *while the s.c.g. was receiving its natural blood supply*; they are consistent with the results obtained by perfusion experiments; they offer justification for the reliability of those experiments; and by extending them our observations serve to support the inferences drawn from them in favor of a chemical step at the ganglionic synapses.

Against the conclusion that a-ch. acts as an agent for nerve impulses in the relay in the s.c.g. Eccles (1936) has laid strong emphasis on the indications that normally the synaptic transmitter disappears very quickly from the ganglion after a pre-g. volley, and also on electrical evidence that eserine does not prevent the quick disappearance. If a-ch. were the transmitter, eserine should protect it from destruction, and one would expect "a prolonged action of the a-ch. liberated by a single preganglionic volley with a consequent repetitive discharge from the ganglion cells, and this does not occur," so Eccles declares. From this evidence he has drawn the conclusion that the quick disappearance is not due to action of a cholinesterase. Thus he has thrown doubt on the concept that a-ch. serves as a chemical mediator in sympathetic ganglia. Observations reported above (see figs. 3, 5 and 6) raise the question as to whether electrical records from the ganglion or from post-g. fibers are more trustworthy than the response of one of the natural indicators of impulses discharged over these fibers—the n.m. As shown in figure 3, prostigmin (equivalent to eserine) may have a remarkable potentiating effect on single pre-g. volleys; because maximal shocks were used, this effect was interpreted as due to repetitive discharges from the ganglion cells. If this interpretation is valid—and it appears unavoidable from the present evidence, strengthened by the data obtained in skeletal muscle (Brown, Dale and Feldberg, 1936; see Rosenblueth and Morison, 1937)—the conclusion may be reached that the synaptic excitatory state, analogous to Sherrington's (1925) c.e.s., may attain supraliminal values.

Eccles (1936) opposes two further objections to the view that a-ch. is the chemical mediator of the nerve impulses at the sympathetic ganglia. He states that there is no mechanism known which could destroy acetylcholine in normal conditions with a rapidity sufficient to prevent repetitive firing in all circumstances. This objection, in our opinion, is not warranted by the data available (see Rosenblueth and Morison, 1937, for a full discussion). Eccles also argues that the facilitation curve of the ganglion is uninfluenced by eserine and that this curve is a measure of the rate of destruction of the synaptic transmitter. If a-ch. were this transmitter, the facilitation curve should be prolonged because eserine protects a-ch. from destruction. He concludes that since this prolongation does not occur a-ch. is not the mediator. Although the argument is sound the conclusion seems to us not valid because it has not been established that eserine does not prolong the period of facilitation. The experiments on which Eccles bases this premise are illustrated in figure 9 of his paper published in 1935. In this figure the scattering of the points from which the facilitation curves are drawn is so great as to render unreliable a quantitative conclusion. The dose of eserine injected in this experiment was not sufficient (cf. p. 223). The order in which the observations were made after eserine is not stated, nor is there any mention of the time employed for making

them. We have shown above (cf. fig. 5) that the action of prostigmin is transitory; the action of the less stable and less potent eserine is still more transitory. Until more accurate data are available and until experiments are made in which the observations are performed shortly after sufficiently large doses of eserine, the effects of this substance on the facilitation curve will not be known.

If the theory is accepted that synaptic transmission occurs solely as a consequence of the electrical influence of one unit on the next, what becomes of all the phenomena, discovered during the past sixteen years, which have been interpreted as evidence for a chemical mediation? Why an appearance of a-ch. during stimulation of pre-g., parasympathetic and motor nerve fibers? Why the mimicking of the action of impulses carried by these fibers, when a-ch. is delivered to the elements on which the impulses act? Why a prolongation of the influence of a-ch. on these elements when it is protected from destruction? These and other similarly pertinent questions are not answered by the theory of electrical transmission. Indeed, that theory leaves a multitude of facts regarding events at synapses and nerve endings, repeatedly observed in recent years, as irrelevant phenomena. The theory of chemical transmission accounts for them and admits the electrical events as well. Until the electrical theory can provide equally satisfactory reasons for the facts at hand, it must be regarded as seriously defective.

#### SUMMARY

1. The known resemblances between sympathetic ganglia and motor plates of skeletal muscles are reviewed, and the methods here used—study of the superior cervical ganglion (s.c.g.) *having a natural blood supply* and influencing the nictitating membrane (n.m.)—are described.

2. Evidence is presented as to relative doses of acetylcholine (a-ch.) which produce stimulant and depressant effects on the s.c.g. (fig. 1).

3. Prostigmin (used instead of eserine) may have a depressant action on the ganglion, but it may clearly potentiate the responses to maximal single shocks applied to the preganglionic (pre-g.) fibers (fig. 3). It has no direct effect on the postganglionic (post-g.) fibers or n.m.

4. Given shortly before tetanic stimulation of pre-g. fibers prostigmin cuts short the contraction and turns it into relaxation; as minutes pass the depressant action gradually disappears (fig. 5). The relaxation is attributed to excess of a-ch., preserved from prompt destruction by prostigmin.

5. If injected after tetanic stimulation of pre-g. fibers has failed to maintain the n.m. in full contraction, prostigmin causes an increase of contraction (fig. 6). If the lower contraction resulted from "fatigue" (i.e., insufficient a-ch. to activate some ganglion cells), the increase is explicable as a preservation of a-ch. until more cells are activated.

6. Injection of a-ch. during the fatigue phase has an effect similar to that produced by prostigmin (fig. 2), and can be explained on the same basis; injected at the start of tetanic stimulation, a-ch., like prostigmin, quickly interrupts contraction and induces relaxation (fig. 2). Again the action, like that of prostigmin (see above, paragraph 4), is attributable to a depressant excess of a-ch. at this phase.

7. Either a-ch. (fig. 7) or prostigmin (fig. 8) has a decurarizing action on the ganglion. In explanation, curare may be regarded as raising the threshold of excitation of the ganglion cells, so that the fatigue effect is magnified (i.e., the level of contraction falls); a-ch., by supplying an extra quantity, and prostigmin, by protecting from destruction the a-ch. which is naturally produced, raise the stimulus above the raised threshold.

8. A brief tetanus, introduced in a series of maximal single shocks, causes the succeeding contractions to be much higher than those which preceded (see fig. 4). Probably the tetanus releases in the ganglia an adjuvant (perhaps potassium) of a-ch., which results in repetitive discharges from single pre-g. volleys.

9. In the discussion the bearing of these observations on the chemical theory of synaptic transmission is considered.

#### REFERENCES

- BACQ, Z. M. *Arch. internat. de Physiol.* **44**: 112, 1936.  
 BARSOUM, G. S., J. H. GADDUM AND KHAYYAL. *J. Physiol.* **82**: 9P, 1934.  
 BRISCOE, G. *Ibid.* **87**: 425, 1936.  
 BROWN, G. L. *Ibid.* **81**: 228, 1934.  
 BROWN, G. L., H. H. DALE AND W. FELDBERG. *Ibid.* **87**: 394, 1936.  
 BROWN, G. L. AND W. FELDBERG. *Ibid.* **88**: 265, 1936.  
 DALE, H. H. AND W. FELDBERG. *Ibid.* **81**: 39P, 1934.  
 DALE, H. H., W. FELDBERG AND M. VOGT. *Ibid.* **86**: 353, 1936.  
 EASSON, L. H. AND E. STEDMAN. *Proc. Roy. Soc., London B* **121**: 142, 1936.  
 ECCLES, J. C. *J. Physiol.* **81**: 8P, 1934.  
     *Ibid.* **85**: 207, 1935.  
     *Ergebn. d. Physiol.* **38**: 339, 1936.  
 ELLIOTT, T. R. *J. Physiol.* **35**: 367, 1907.  
 FELDBERG, W. AND J. H. GADDUM. *Ibid.* **81**: 305, 1934.  
 FELDBERG, W. AND A. VARTIAINEN. *Ibid.* **83**: 103, 1934.  
 LANGLEY, J. N. *Ibid.* **27**: 224, 1901.  
     *Ibid.* **36**: 347, 1907.  
 LANGLEY, J. N. AND H. K. ANDERSON. *Ibid.* **19**: 131, 1895.  
     *Ibid.* **31**: 365, 1904.  
 ORIAS, O. *This Journal* **102**: 87, 1932.  
 ROSENBLUETH, A. AND W. B. CANNON. *Ibid.* **99**: 398, 1932.  
 ROSENBLUETH, A., H. DAVIS AND B. REMPEL. *Ibid.* **116**: 387, 1936.  
 ROSENBLUETH, A., D. B. LINDSLEY AND R. S. MORISON. *Ibid.* **115**: 53, 1936.  
 ROSENBLUETH, A. AND R. S. MORISON. *Ibid.* **119**: 236, 1937.  
 SCHIFF, J. M. *Muskel- und Nervenphysiologie*. Lehr, 1858, p. 189.  
 SHERRINGTON, C. S. *Proc. Roy. Soc., London B* **97**: 519, 1925.  
 SIMONART, A. AND E. F. SIMONART. *Arch. internat. de Pharmacodyn. et de Thérap.* **49**: 302, 1935.

## CURARIZATION, FATIGUE AND WEDENSKY INHIBITION

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Recent experiments of Dale, Feldberg and their collaborators (see Brown, Dale and Feldberg, 1936) render the view quite likely that the liberation of acetylcholine is a crucial step in the transmission of motor nerve impulses to skeletal muscle. The data available on the effects of acetylcholine and of eserine—which protects the acetylcholine normally liberated—on the responses of mammalian muscles to indirect activation are apparently contradictory. Thus, acetylcholine may depress muscular contractions when injected before or after eserine, without previous curarization (Rosenblueth, Lindsley and Morison, 1936; Briscoe, 1936c), but leads to a potentiation when administered after curare (Rosenblueth, Lindsley and Morison, 1936; Briscoe, 1936c; Wilson and Wright, 1936). Similarly eserine (or prostigmin) which, as is well known, has a decurarizing action (Pál, 1900) may either depress (Rosenblueth, Lindsley and Morison, 1936) or augment (Brown, Dale and Feldberg, 1936) the muscular responses to motor nerve impulses.

The original purpose of the present study was the explanation of these apparent discrepancies. The experimental results obtained, however, suggested the extension of this purpose to include an attempt to explain the problems of muscular function mentioned in the title in terms of the concept of chemical mediation of the motor nerve impulses. This second purpose became the central interest of the study.

**METHOD.** Cats were used, under dial anesthesia. The majority of the observations were made on the gastrocnemius-soleus muscle; some experiments performed on the tibialis anticus yielded similar results, with only minor quantitative differences. A cannula was inserted into the trachea for artificial respiration, when necessary. The sciatic nerve was cut on one or both sides, and either the popliteal or the peroneal branch was dissected for purposes of stimulation or recording. The stimulating electrodes were usually shielded silver wires. The same electrodes were employed to lead off the electric responses of the nerves to the recording system. On a few occasions fluid silver-silver chloride-Ringer electrodes were also used for stimulation. One, two or three pairs of electrodes were applied to the peripheral portion of the nerves. When the muscles were stimulated directly, steel or silver-silver chloride needles were inserted into their

bodies and tendons. The muscular electric responses were led to the amplifier either through these silver needles, or by means of concentric electrodes of the Adrian-Bronk type, or, finally, through bipolar silver-silver chloride wires, insulated except at the tip, and with an interpolar distance of less than 1 mm.

One or both legs were fixed by drills in the tibia. The contractions of the freed tibialis anticus or gastrocnemius-soleus muscles were recorded on a kymograph by attaching the tendon to the short arm of a writing lever pulling against strong rubber bands. The magnification was 6- to 10-fold. The shortening of the muscle was usually less than 1 cm. The initial tension was 100 to 500 grams. The records obtained were practically linearly proportional to the tensions developed. In many of the experiments in which the electric responses were studied the muscles were attached to a stiff steel-spring myograph. The shortening was then less than 1 mm.

The stimuli used varied with the purposes of the experiment. Occasionally a Harvard induction coil with 5 volts in the primary circuit was employed. Single stimuli were usually discharges from condensers of 1.0 to  $0.01 \mu$  F capacity, the total resistance in the circuit being approximately  $5000 \omega$ ; they were applied at regular intervals by hand or by means of a suitable mechanical interruptor. Repetitive stimulation at high frequencies was obtained from a multivibrator circuit or from the directly amplified pulses of a photo-electric cell illuminated at the desired frequency through rotating disks driven by a motor at 30 revolutions per second. These direct-current pulses were passed through a transformer, to render them diphasic, before they were applied to the nerves. The stimuli were invariably maximal.

The electric responses were led to a 5-stage capacity-coupled amplifier; the frequency characteristic of the amplifier was linear from 1 to 10,000 c.p.s., less 2 per cent distortion at full output. A cathode-ray oscillograph or a loud-speaker permitted the amplified signals to be recorded, seen or heard.

The injections were usually made into a jugular vein. In some cases, mentioned in the text, intra-aortic injections led the substances more directly to the muscles. The drugs employed were: eserine (physostigmine salicylate, Burroughs Wellcome), prostigmin (Roche), atropine sulphate (Merek) and curare (the Brazilian crude product). The effects of physostigmine and prostigmin were qualitatively identical with some quantitative differences; the two drugs will therefore be indiscriminately referred to by the term eserine.

**RESULTS.** 1. *Normal and fatigued muscles.* Stimulation of the popliteal nerve for relatively long periods (e.g., 5 min.) at tetanizing frequencies (30 or more per sec.) leads, as is well known (see Hofmann, 1903a) to an initial rise of tension of the gastrocnemius-soleus, succeeded by a slow fall, until a fairly steady level is reached. The higher the frequency, the lower

this steady level. In five animals out of seven, in which the electric responses of normal muscles during tetanic stimulation were studied, repetitive firing of the muscle to some of the stimuli was observed. This repetitiveness was present only early in the series, though not till a few stimuli had been delivered (fig. 1A). The optimum frequency was 30 to 120 per second; below 30 per second repetitiveness was only rarely encountered; at frequencies higher than 120 per second the spike potential from a given volley is just over when the next volley arrives, thus masking possible repetitiveness. The spike potentials of the muscle showed variations in

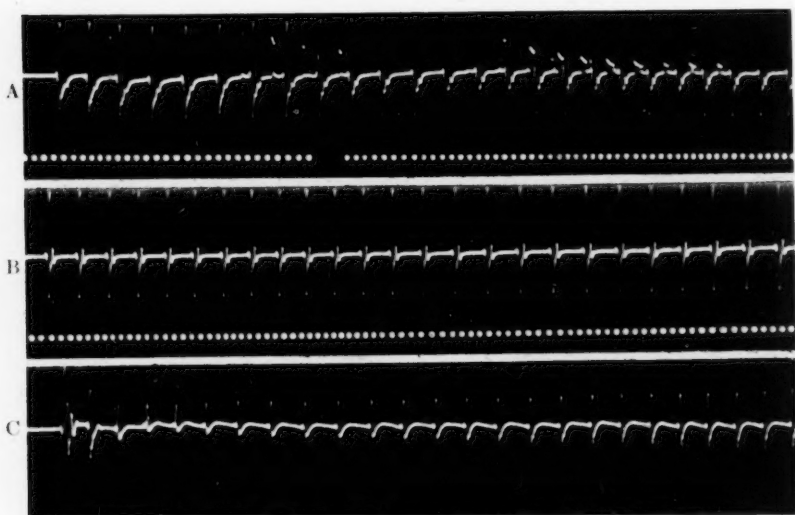


Fig. 1. Repetitive discharges of normal and eserinizd muscle to single nerve volleys during tetanic stimulation. Concentric needle electrodes on gastrocnemius. Popliteal nerve stimulated at the rate of 30 per second. In this and the succeeding records of electrical responses the small intervals in the time-signal record 10 msec.

- A. Before eserine. Beginning of tetanus.
- B. Twenty seconds later.
- C. After eserine. Beginning of another similar tetanus.

magnitude parallel to the changes in tension, since they decreased as the tension decreased (Davis and Davis, 1932).

Slowing the rate of stimulation during the steady level may lead to an increase of tension, whereas accelerating the rate produces a fall of tension (Wedensky, 1891; Hofmann, 1903a). Indeed, merely single shocks through a pair of electrodes on the nerve near the muscle, while the repetitive stimulus is continuously applied to a far pair, cause a transient fall of tension (fig. 2A). Direct stimulation of the muscle during the steady level, on the other hand, leads to a further contraction, usually succeeded by a transient subsequent fall. If single shocks are thus applied directly to the

muscle and an intensity is used which yields maximal twitches when the nerve is not simultaneously activated, the contractions superimposed on the steady level during continuous stimulation of the nerve are usually only a small fraction of the maximal twitch. In an experiment reported by Davis and Davis (1932) the tension during tetanic stimulation had fallen to 20 per cent of its initial value, while the superimposed twitches from direct stimulation of the muscle were only 14.5 per cent of the maximal twitches. Davis and Davis concluded that the latter value indicates that at most only 14.5 per cent of the muscle fibers could have been idle

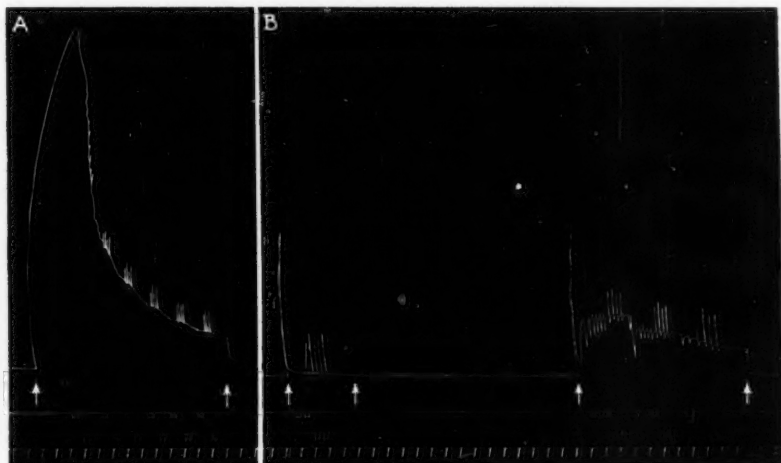


Fig. 2. Opposite effects of single induction shocks delivered to nerve and muscle during tetanic stimulation. Two pairs of electrodes on the popliteal nerve, far and near, respectively, in relation to the muscle. Two silver, silver-chloride needles inserted into the gastrocnemius-soleus muscle. The tetanic stimuli were delivered through the far electrodes; the period of stimulation is marked by arrows. Upper signal: single induction shocks to near electrodes on nerve. Middle signal: single shocks to muscle. In these and the succeeding kymograph records, unless otherwise stated, the lowest signal marks 5-second intervals.

A. Before physostigmine. Tetanus: 120 per second.

B. After physostigmine (1 mgm. per kgm.). Tetani: 60 and 30 per second.

during the tetanic stimulation, and that the decrease of tension and of action potential occurs, therefore, in the active individual fibers.

A stimulus applied directly to a resting muscle may activate both nerve and muscle fibers. A shock which is maximal in such conditions may not be maximal when the possible participation of the nerves is eliminated. In the observations mentioned single shocks, maximal to the resting muscle and delivered during the steady level, induce a certain tension. Strengthening the single shocks at this point, however, leads to increased contractions until the ratio of these superimposed twitches to the maximal

twitches approximates the ratio between the tension at the steady level and the initial maximal tension for the corresponding frequency. It is therefore possible to account for the fall of tension during tetanus in terms of idle muscular fibers, although the nerve volleys remain maximal throughout.

Injections of acetylcholine or potassium chloride (cf. Cowan, 1936b) during the steady level of contraction lead to a prompt, marked, transient fall of tension (figs. 3 and 5A.) The fall from large doses of acetylcholine

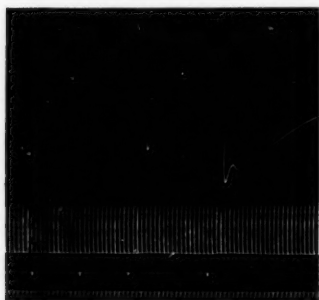


Fig. 3

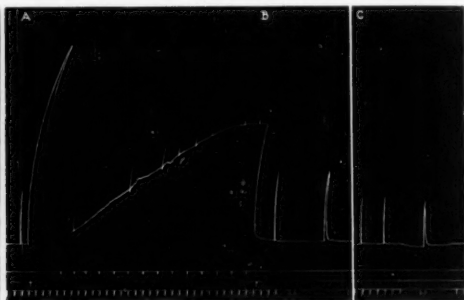


Fig. 4

Fig. 3. Depression of tetanic response by acetylcholine. Adrenals ligated; atropine (1 mgm. per kgm.). Upper record: left gastrocnemius-soleus stimulated indirectly throughout at the rate of 30 per second. Lower record: right gastrocnemius-soleus; maximal twitches from indirect stimulation. The resting level of the two muscles was the same. Upper signals: intravenous injections of 1, 2.5, 5 and 10 mgm. acetylcholine.

Fig. 4. Post-tetanic increase of maximal twitches in the normal gastrocnemius-soleus muscle.

A. Single twitch (slightly potentiated by a previous tetanus) and tetanic stimulation at 240 per second through a far pair of electrodes on the nerve (middle signal). The upper signal denotes single shocks through a near pair of electrodes during the tetanus.

B. Slow and fast records of twitches after the tetanus.

C. Slow and fast records of twitches 3 minutes later.

is usually complex, an initial sharp effect being succeeded by a slow prolonged return (fig. 3).

When high frequencies of indirect stimulation (e.g., 240 per sec.) are employed, the responses of normal skeletal muscles are complex (fig. 4). An initial sharp, high rise is promptly followed by a fall of tension. If the stimulus is continued, a slow persistent rise appears which increases for several minutes and is finally succeeded by an even slower decline (see Hofmann, 1903a; Cowan, 1936a).

Observations of the action potentials of the A fibers in the popliteal

nerve at this frequency of stimulation show full-sized spikes for a few seconds. After this some alternation is apparent which becomes progressively more marked. This alternation coincides, therefore, with the phases of fall and slow rise of tension in the mechanogram. That this relative failure of the nerve is not responsible for the mechanical sequence, however, is indicated by the inability of single shocks delivered through a near pair of electrodes on the nerve to elicit, consistently, muscular responses (fig. 4). Electrical records of the muscle indicate that the occasional twitches which these single shocks evoke are produced by a momentary slowing of the frequency of stimulation, resulting because the rhythm of the repetitive stimuli is interrupted by the refractory period which succeeds the single shocks.

The action potentials of the muscle during high-frequency stimulation differ from those of the nerve; alternation appears in the muscle almost from the start and is always much more marked than in the nerve. The recurring patterns of alternation in the nerve and the muscle do not usually have the same rhythm; thus, the nerve may show rhythmically recurring cycles which last 33 msec. (i.e., every 8th shock), while the muscle may show cycles of 66 msec. On this background of alternating muscular action potentials parallel quantitative variations of mechanogram and electrogram are frequently recognizable—i.e., the tension decreases and increases with the electric responses.

Injections of acetylcholine or potassium chloride have similarly different effects, depending on the dose of the substance and the period of the response at which the injection is made. These differences may be summarized in the statement that during the initial fall the effects are mainly depressant, during the ultimate decline they are mainly stimulant, and during the intermediate secondary rise they are mixed, first an increase, then a fall, finally another increase. Within limits larger doses tend to have greater depressant action, and smaller doses have greater stimulant effects. Figures 3, 5 and 11A illustrate typical instances. The muscle action-potentials show variations of magnitude parallel with the mechanical changes.

Tetanic stimulation of a muscle through its motor nerve leads to changes in the responses to single shocks which may last for several minutes. Thus, if a series of single shocks is delivered to a nerve at intervals of 5 or 10 seconds and a tetanus (e.g., of 30–120 per sec. for 10–30 sec.) is given through a pair of far electrodes the subsequent twitches undergo typical changes. Immediately after the tetanus they are smaller than the original ones; they then increase in magnitude, but mainly in duration, until they become greater than the original twitches; then they subside slowly to the initial level (see Schiff, 1858; Hofmann, 1904). This effect, slight in fresh muscles, is usually more marked in fatigued preparations, after several contractions have been elicited (cf. Guttman, Horton and Wilber, 1936).

Figure 4 illustrates a typical instance of a post-tetanic increase of responses to single shocks in an unfatigued muscle.

In order to test whether the increment is a consequence of repetitive discharges in the muscle or a consequence of a contracture, records of the electric responses of the muscle were taken, before and after a tetanus. It was expected, *a priori*, that only small changes could be present and only in some of the fibers, for the increments in tension were not very large in the fresh muscles (30 per cent or less). A further difficulty appeared when the study was made. The muscles frequently showed contraction-remainders (see Gasser, 1930) after the tetanus, which lasted up to several minutes. Invariably the spike-potentials of the muscle were considerably decreased during these contraction-remainders, although the mechanical responses

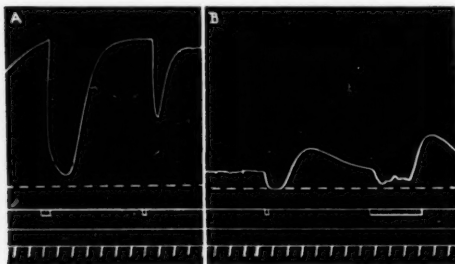


Fig. 5

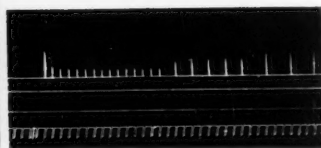


Fig. 6

Fig. 5. Depressant and stimulant effects of potassium on tetanic responses. The upper signals denote intra-aortic injections. The broken line shows the resting level of the muscle.

A. Continuous stimulation at 30 per second. Injections of 30 and 20 mgm. KCl.

B. Continuous stimulation at 240 per second. Two injections of 10 mgm. KCl.

Fig. 6. Wedensky inhibition manifested by twitches after a small dose of curare. Single maximal shocks delivered to nerve as indicated by the responses.

showed a clear increase in magnitude and duration; possible repetitive discharges would probably be similarly affected. Favorable instances in which highly localizing bipolar electrodes were used to lead the electric responses to the recording outfit, and in which the contraction-remainder was slight or absent, furnished records which may be interpreted as denoting that the muscle was making a tetanic response to single shocks during the post-tetanic augmentation period. Figure 7 illustrates one of these favorable instances. In a complex record from a large number of muscle fibers it is very difficult to distinguish repetitive responses from temporal dispersion. This difficulty was encountered here and in the observations on tetanic stimulation described above (fig. 1). Only studies of a single fiber would be decisive in this regard. Since such studies were not made the present conclusions are necessarily tentative. The analogous post-

tetanic increase in the superior cervical ganglion (Cannon and Rosenblueth, 1937), where repetitive discharges appear to be the only explanation of the phenomenon, lends support to these conclusions. The difficulty of interpretation just mentioned does not apply to the results obtained after injections of eserine (figs. 1 and 9) because if the large and prolonged after-discharges recorded were due to temporal dispersion the first spike would be considerably smaller, and this is not the case.

II. *Eserine*. Injections of physostigmin or prostigmin during continuous stimulation of the motor nerves produce effects which depend on the doses employed and on the frequency of stimulation.<sup>1</sup> With slow frequencies (e.g., 1 per 10 sec.) 1 mgm. per kgm. of physostigmin or 0.2 mgm. per kgm. of prostigmin leads to a marked increase of the mechanical responses (Brown, Dale and Feldberg, 1936). The same doses of the drugs, injected while the muscles are activated indirectly at higher frequencies—e.g., 5 per second—lead to a depression of the contractions (fig. 8). Larger doses of the drugs, with similar frequencies of stimulation, tend to diminish the

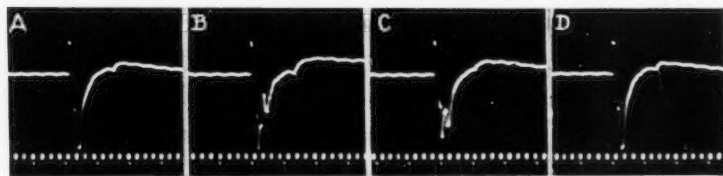


Fig. 7. Post-tetanic repetitive electrical responses of muscle to single maximal nerve volleys. Isometric contraction.

A. Before the tetanus.

B, C and D. Thirty, 60 and 120 seconds after tetanic stimulation at 240 per second (20 sec.).

increase and augment the depression. The general statement may therefore be made that, within limits, the larger the dose and the faster the frequency, the greater the depression obtained.

As Brown, Dale and Feldberg (1936) showed, the potentiated responses to single shocks after eserine are no longer twitches, but brief tetani; a single nerve volley—as confirmed by electrical records—gives rise to repetitive muscle action-potentials (figs. 1 and 9).

Repetitive stimulation after eserine (e.g., physostigmin, 1 mgm. per kgm.) yields responses which vary with the frequency employed. Very slow frequencies (e.g., 1 per 10 sec.) elicit potentiated short tetani, as mentioned above. With frequencies between 1 and 5 per second, which do not lead to significant fusion of the mechanical responses, a sequence of contractions ensues in which first a decline and later a rise are recognizable (cf. Rosenblueth, Lindsley and Morison, 1936). The electric responses show similar variations—there is first a decrease and later an increase in the

<sup>1</sup> A preliminary report of these observations appeared in *Science* 84: 551, 1936.

magnitude of the first spike-potential; the repetitiveness, on the other hand, rapidly disappears so that the last responses in the series closely resemble those obtained without eserine (fig. 9).

As the frequency of stimulation is increased (e.g., 10 to 30 per sec.) the same sequence is observed in the partially fused responses, but the late rise is more prominent. From 60 to 100 per second, as a rule, a typical picture of Wedensky inhibition results (fig. 2B; see Cowan, 1936a; Briscoe, 1936a

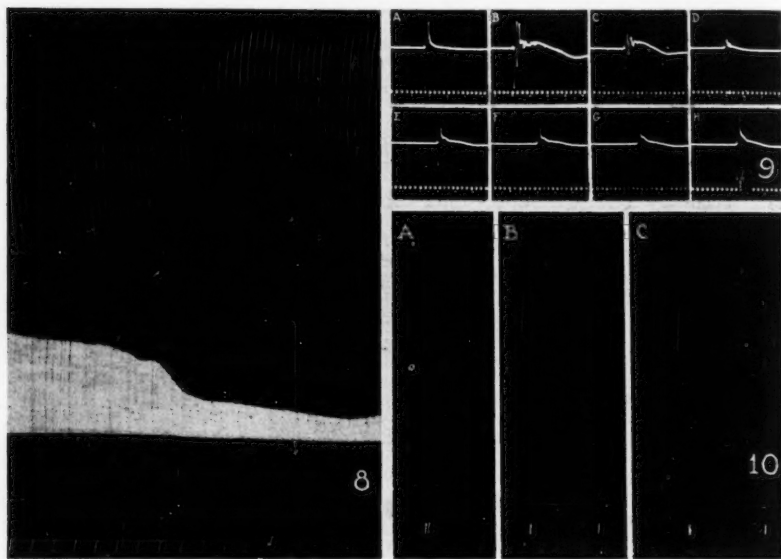


Fig. 8. Upper record: right gastrocnemius-soleus stimulated indirectly by maximal shocks at a frequency of 1 per 10 seconds. Lower record: left gastrocnemius-soleus stimulated indirectly by maximal shocks at a frequency of 1.3 per second. At signal: physostigmine, 0.6 mgm. per kgm. Time-signal: 30 second intervals.

Fig. 9. Influence of prostigmin on the electric responses of muscle.

A. Single maximal volley before prostigmin.

B to H. After prostigmin. Responses to a series of maximal shocks delivered to the nerve at a frequency of 2 per second.

Fig. 10. Post-tetanic contractions after eserine (physostigmine, 1 mgm. per kgm.). Between signals stimulation of nerve at 180 per second.

and b). High frequencies (e.g., 120-240 per sec.) elicit usually a sharp rise, not as high, however, as that occurring at 30 per second, then a prompt fall to zero, next a slower rise and finally a slow decline. The action potentials show variations parallel with those of the mechanical responses.

When additional single shocks are delivered to the nerve through a pair of electrodes near the muscle, while a tetanic frequency is applied through a far pair, a sharp fall in tension is the rule, in striking contrast with the further contractions which direct stimulation of the muscle elicits (fig. 2).

The main differences between these responses and those evoked from normal muscles by the corresponding frequencies are the following. In eserinizated muscles single shocks elicit tetani, instead of the normal twitches. The normal "Treppe" is replaced by a decline. This decline is evidence of Wedensky inhibition, which occurs at frequencies which in normal muscles lead to well-sustained responses.

When a short tetanus (e.g., 0.5 to 3 sec.) is applied at a high frequency (e.g., 120-240 per sec.) after eserine, the high tension evoked by the stimuli is sometimes succeeded by a small, relatively long subsequent contraction (fig. 10). The study of the electrical responses of the muscle during these mechanical effects demonstrates a series of irregular, asynchronous action potentials during the delayed contraction. This phenomenon was ob-

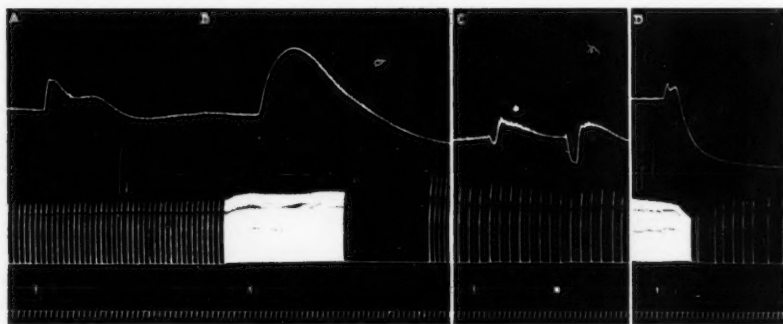


Fig. 11. Depressant and stimulant effects of acetylcholine and eserine. Same animal and records as in figure 3. The resting level of the left gastrocnemius-soleus is shown by the horizontal underlying the upper record. Stimulation of both popliteal nerves; left (upper record), 240 per second; right (lower record), as denoted by twitches.

- A. Acetylcholine: 2 mgm.
- B. Prostigmin: 0.08 mgm. per kgm.
- C. Acetylcholine: 0.1 and 0.25 mgm.
- D. Additional dose of prostigmin, as in B.

tained both after physostigmin and prostigmin. It is by no means general, however; it was encountered in 3 out of 6 animals especially tested in this regard. The data are not sufficient to state what conditions favor its appearance.

The effects of acetylcholine, after eserine, are qualitatively similar to those occurring in normal, non-eserinized muscles (fig. 11). The depressant action in the unfatigued condition is particularly prominent. The effects of potassium ions are also similar to those reported in section I.

The effects of a tetanus on the responses to single shocks is more complex after eserine than in the normal muscles. Tetanic stimulation may result in a depression of the twitches which may first be almost complete, and is succeeded by a slow recovery (fig. 12B). In other instances,

however, a marked post-tetanic increase is observed, more striking even than that which occurs in the normal or fatigued muscles (fig. 12A). The electrical records clearly demonstrate the tetanic nature of these increased post-tetanic responses to single volleys.

III. *Curare*. The action of small doses of curare on the muscular responses to indirect repetitive stimulation has been extensively studied. The first striking change which the drug produces is that Wedensky inhibition occurs at relatively slow frequencies of stimulation (Hofmann, 1903a). Bremer and Titeca (1935), working with frog muscles, reported typical Wedensky inhibition even with frequencies as low as 10 per second. In the present experiments series of twitches elicited 100 times slower—i.e., at 10-second intervals—showed a marked decline (fig. 6), quite comparable with the decline of the tetanic response when more frequent stimuli are employed.

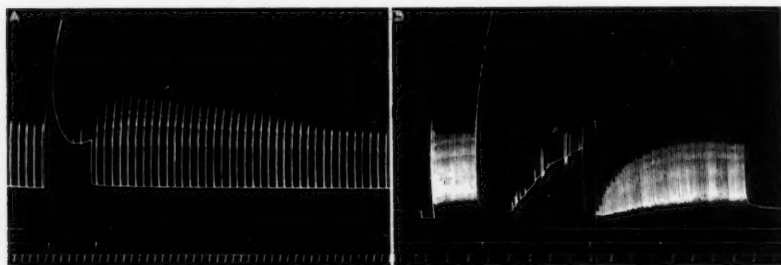


Fig. 12. Post-tetanic increases and decreases of twitches after eserine. Single maximal shocks continuously delivered to the nerves at frequencies shown by the records. Between the signals 240 per second were applied through a far pair of electrodes.

A. After prostigmin (0.15 mgm. per kgm.).

B. In another animal, after prostigmin (0.5 mgm. per kgm.).

With slightly larger doses of curare a condition is reached in which single nerve volleys fail to stimulate the muscle, but repetitive activation of the nerve induces contraction (Hofmann, 1903b). For purposes of the present report the stage of curarization described in the preceding paragraph will be called the first stage; the stage at which repetitive stimulation becomes necessary will be called the second stage; finally, complete curarization, when no responses may be obtained by nerve stimulation, will be called the third stage.

As reported by Boyd (1932), a short tetanus (2 to 60 sec.) at high frequency (120 to 240 per sec.) temporarily decurarizes the muscle. In this decurarization the three stages mentioned appear in a reversed order. The phenomenon is very striking when a fully curarized muscle gives clear twitches in response to single shocks for a few minutes after a tetanus has been applied for a few seconds. Within limits the duration and degree of

the decurarization depend on the duration and the frequency of the tetanus employed.

Injections of acetylcholine (Rosenblueth, Lindsley and Morison, 1936; Briscoe, 1936c) or of potassium salts (Wilson and Wright, 1936) have decurarizing effects quite similar to those obtained from tetanization.

The antagonism between curare and eserine is well known (for references see Rosenblueth, Lindsley and Morison, 1936). It is interesting to encounter this antagonism as regards Wedensky inhibition, although both substances favor the appearance of this depression. A dose of eserine which alone would induce the Wedensky phenomenon during tetanic stimulation (at a frequency, for example, of 60 per sec.) abolishes it in a muscle at the first stage of curarization at the same frequency of stimulation. Conversely, a dose of curare, which would by itself lead to Wedensky inhibition, abolishes the inhibitory effects of eserine (fig. 13; see Cowan, 1936a; Briscoe, 1936a and b).

Wilson and Wright (1936) reported that eserine augments the decurarizing action of potassium ions. An attempt was made in the present experiments to confirm this report and to observe also whether eserine augments the decurarizing action of a tetanus. Although an apparent enhancing action of eserine was sometimes recorded, the interpretation of the results is difficult because of the disinhibitory effects of eserine after curare, reported above. When test single shocks were applied at 15-second intervals, which eliminated any Wedensky inhibition, this enhancing action of eserine did not occur.

**DISCUSSION.** A. *The transmission of the motor nerve impulses.* Since knowledge of chemical transmission at the neuromuscular junction is recent and still not generally accepted, it is pertinent to discuss the foregoing results from this standpoint.

Before proceeding with the discussion it may be mentioned that the possibility that some of the experimental results could be due to vascular changes in the muscles was considered and discarded for the following reasons. The same substance, e.g., eserine, acetylcholine, potassium or curare, may elicit opposite effects, depending on the experimental conditions during which it is injected. The vascular effects would in all probability be uniform for a given substance. The majority of the results reported are too marked to be reasonably attributed to vasomotor changes. In a few experiments the blood pressure was recorded and no significant or consistent changes were observed which could be correlated with the muscular phenomena.

A prominent feature of several of the results obtained in the present study is their prolonged duration. The after-discharge of the eseritized muscle to single nerve volleys (fig. 9), the inhibitory effects of a single twitch on the succeeding contraction for 10 seconds after curare (fig. 6), the post-tetanic potentiation of twitches in normal muscles (fig. 4) or after curare, which may last several minutes, are all facts which challenge

explanation. After-discharge of eserinizd denervated muscles does not occur (Brown, Dale and Feldberg, 1936). Direct stimulation of a fully curarized muscle does not show Wedensky inhibition. These phenomena occur, therefore, at the neuromuscular junction. Quite obviously the classic concept that all the nerve impulse does to the muscle is to apply a brief electrical stimulus is inadequate to explain these facts. The theory that chemical mediators are involved in the transmission process furnishes a satisfactory basis for the explanation sought.

Since eserinizd muscle fibers respond repetitively to single nerve volleys, it may be concluded that the excitatory process at the neuromuscular junction can attain supraliminal values. In a recent review Eccles (1936) suggests that the repetitiveness of the muscular response is due to a hyperexcitability of the motor plate produced by eserine, not to an enduring synaptic transmitter. In support of this suggestion he adduces the fact that eserine causes fibrillary twitches in the muscle. The relevance of this argument is questionable. A mere change of excitability will not induce either twitches or tetani unless there is an adequate stimulus. The stimulus of the fibrillary twitches is unknown. As pointed out by Eccles, the theory of electrical neuromuscular transmission provides no clue to their origin. The chemical theory, on the contrary, suggests the possibility of a leakage of acetylcholine as the cause, a possibility which is in accord with the absence of fibrillation after degeneration of the nerves (Langley and Kato, 1915).

As both the mechanical and the electrical records show (figs. 8 and 9; Dale, Brown and Feldberg, 1936), a single nerve volley after eserine sets up a tetanus which is independent of the fibrillation—i.e., the nerve volley not only elicits a first muscular discharge succeeded by a refractory period, but also other discharges which may continue for as long as 60 msec. The problem on hand is the nature of the stimulus set up by the nerve volley which is responsible for the prolonged response. Neither the action potential of the nerve, nor the hypothetical "detonator" postulated by Eccles—but not defined for skeletal muscle—possesses the duration necessary for these prolonged effects. The suggestion of Eccles appears, therefore, to evade the issue. Furthermore, any explanation suggested for the action of eserine should account not only for potentiation at slow frequencies of stimulation, but also for depression at fast frequencies (figs. 2 and 8). The suggestion of Eccles does not meet this test, whereas the explanation offered by Brown, Dale and Feldberg (1936) and developed below accounts for all the data at hand.

Since acetylcholine is released at neuromuscular junctions (Dale, Feldberg and Vogt, 1936) and is also able to set up conducted disturbances in muscle (Brown, Dale and Feldberg, 1936), it appears reasonable to attribute the after-discharge on single nerve volleys after eserine to the persistence of the acetylcholine released by the nerve impulses at the junctions

(Brown, Dale and Feldberg, 1936). An excess of acetylcholine is known, however, to paralyze muscles, instead of exciting them (see Dale, Feldberg and Vogt, 1936; Rosenblueth, Lindsley and Morison, 1936). On the other hand, there is a threshold concentration of acetylcholine, below which no excitation occurs, as would *a priori* be expected, since it is here setting up an all-or-none response. From the repetitive discharges it may therefore be concluded that the quanta of acetylcholine liberated per nerve volley in the experimental conditions under discussion are not just threshold, but well supraliminal. Figure 14 illustrates these conclusions schematically.

Eccles (1936) objects to the view that acetylcholine is the neuromuscular mediator because of the lack of provisions for its rapid removal in the normal muscle. He deems the cholinesterase insufficient, for the following reason. Easson and Stedman (1936) reported that each active center of the enzyme destroys in the optimum conditions 1490 molecules of acetylcholine per second. Eccles calculates therefrom that in the period of 1 msec., during which the synaptic transmitter acts, each active center could only destroy 1 or 2 molecules of the ester. He then assumes that there cannot be enough esterase in the muscles to destroy enough acetylcholine to prevent repetitive firing to single nerve volleys in normal conditions. Since this repetitive firing does not occur he concludes that acetylcholine cannot be the transmitter. For this argument to have any significance it would be necessary to know how many molecules of acetylcholine are necessary to activate the muscle fibers and how much esterase is actually available at the neuromuscular junctions for the destruction. These questions are for the present unanswerable.

It is premature to attempt to hypothecate the mode of action of acetylcholine at the neuromuscular junction. Lapicque (1936) has suggested that its only rôle is to potentiate within the muscle the effects of the electric stimulus which the action potential of the nerve impulse delivers. This suggestion is unsatisfactory, for injected acetylcholine sets up conducted disturbances without any concomitant nerve impulse—i.e., without any concomitant electrical stimulus (Brown, Dale and Feldberg, 1936).

Although a slow destruction of acetylcholine when protected by eserine can account for the repetitive effects of a single nerve volley, obviously this explanation fails to explain satisfactorily the more prolonged effects reported above. It is inconceivable, for example, that a persistence of acetylcholine could occur without eserine for the long periods during which the post-tetanic increases of response may be observed (pp. 241, 246). Such effects lead, therefore, to the inference that other persistent chemical changes result directly or indirectly from the nerve impulses. Bringing to bear on the problem the analogy with transmission at the sympathetic ganglia, it can be tentatively assumed that mobilization of potassium ions (cf. Brown and Feldberg, 1936a) may be the factor responsible for the more persistent changes.

B. *Wedensky inhibition*. It is customary to attribute to Wedensky inhibition all the cases in which the response of a muscle to persistent repetitive stimulation follows or approximates the course described by Wedensky—i.e., a single twitch at the start of stimulation succeeded by subsidence to inactivity. This custom has been followed thus far in the present report. It is important to recognize, however, that even if the gross behavior is similar the mechanism underlying this behavior may be entirely different for different experimental conditions. Lucas (1911) in his classical work on Wedensky inhibition suggested a single explanation for the inhibition which appears when a nerve is narcotized and that which occurs in fatigued muscles. This single explanation was that subnormal impulses, incapable of activating the muscle, were capable of establishing a refractory period and thus rendering the subsequent impulses equally subnormal. The experimental basis for this assumption was that the inhibition appeared only when the stimuli were applied within the refractory period of the nerve. The interpretation of Lucas has been shown to be inadequate, because the nerve impulses do not traverse the narcotized region of the nerve (Kato *et al.*, 1929), and because Wedensky inhibition occurs after curare (Bremer and Titeca, 1935; fig. 6) or eserine (figs. 2 and 13) at frequencies which exclude the refractoriness set up by the previous impulses.

From the data reported here the conclusion may be reached that there are at least two quite different conditions of the neuromuscular system which lead to the Wedensky phenomenon, for the following reasons: 1, if curare and eserine produced Wedensky inhibition by a single mechanism their effects should sum, instead they cancel (fig. 13; Cowan, 1936a; Briscoe, 1936a and b); 2, acetylcholine and potassium injected during Wedensky inhibition produce further depression after eserine, while they dis-inhibit the responses which occur after curare (pp. 241, 247). The classical Wedensky inhibition of fatigued systems behaves like that which occurs after curare in these respects—eserine, acetylcholine and potassium increase the responses. In normal unfatigued muscles, on the other hand, these substances produce or emphasize the Wedensky effect (figs. 3 and 5).

The schema in figure 14 affords a simple basis for understanding the two different conditions in which repetitive nerve impulses will first stimulate, then fail to elicit contraction. The assumption is now made that when repetitive stimuli are applied the quanta of acetylcholine released per nerve impulse decrease progressively; this assumption will be discussed more fully in section D. If such a decrease occurs it is reasonable to expect it to be more marked the greater the frequency of stimulation. Wedensky inhibition in a fatigued preparation is then explicable as the result of a rapid decline of the amounts of acetylcholine liberated by successive nerve volleys, so that having started within the effective range they soon become subthreshold in concentration for some fibers. Injections of acetylcholine would then add to the concentration, raising it again to the effective range; this is confirmed experimentally (see fig. 11A).

After eserine the inhibition observed at relatively slow frequencies of stimulation would be due to the accumulation of the protected acetylcholine until it reaches a paralyzing concentration. Injections of acetylcholine should then depress the muscle further. The experimental data support this inference (p. 245). When high frequencies are used the decline of the quanta of acetylcholine released will be more rapid than with slow frequencies (see p. 250). After injections of eserine two antagonistic tendencies will therefore coexist—an accumulation of the liberated acetylcholine because of the protective action of eserine, and a decrease of con-

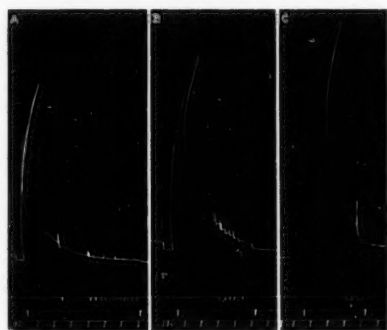


Fig. 13

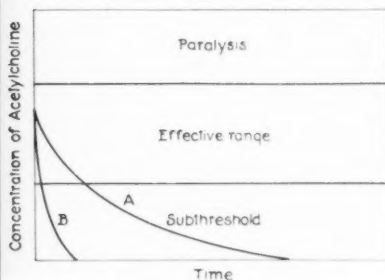


Fig. 14

Fig. 13. Disinhibitory action of curare after eserine (physostigmine, 1 mgm. per kgm.). Between middle signals 120 shocks per second delivered to nerve. The upper signals denote single induction shocks applied through a near pair of electrodes.

- A. Before curare.
- B. After 0.01 cc. curare.
- C. After an additional dose of 0.01 cc. curare.

Fig. 14. Schematic representation of the action of acetylcholine in muscle. The curves illustrate the time-course of destruction of a quantum of acetylcholine released by a nerve impulse: B, before, and A, after eserine. Enough acetylcholine would remain in A, unlike B, to reactivate the muscle after it ceased being refractory.

centration because of the decline of the quanta. The complex curves of tension obtained experimentally (figs. 2B and 12B) are readily accounted for on this basis; the early fall of tension could be due to accumulation and paralysis; the subsequent rise could be due to the decline of the quanta so that the concentration of acetylcholine decreases into the effective range; the final slow fall of tension is explicable as the consequence of a continuing decline of the quanta into the subthreshold zone. The opposite effects of acetylcholine, depressing in the early phases and enhancing later (p. 245), confirm the explanation suggested.

Eccles (1936) objects to the view that the Wedensky inhibition recorded

after eserine is caused by the accumulation of acetylcholine and offers instead the explanation that this depressant action of eserine may be due to a partial "curarization". If this explanation is interpreted as stating that eserine depresses because it produces effects similar to those of curare, i.e., a depression, it becomes mere tautology. On the other hand, if the statement implies that the depression after eserine is due to a mechanism similar to that by which curare leads to Wedensky inhibition, it is wrong, as was shown above (p. 250).

The responses to high-frequency stimulation of normal, unfatigued muscles (fig. 4) are similar to those obtained after eserine with somewhat slower frequencies. The effects of acetylcholine at the different phases of the responses are also analogous. It may therefore be concluded that the underlying mechanism is the same—i.e., that in normal muscles it is possible to accumulate acetylcholine by sufficiently rapid stimulation until the paralytic concentrations are reached in some of the fibers. This conclusion is supported by the repetitive discharges to single volleys which occur early during tetanic stimulation (fig. 1)—a necessary intermediate step before paralysis.

The two types of Wedensky inhibition described may be distinguished as being due to a superabundance or an insufficiency of the chemical mediator. Obviously the Wedensky inhibition which occurs after narcotization of the nerve trunk should be ascribed to another different mechanism, depending not on the processes which occur at the neuromuscular synapse, but on some property of the nerve—i.e., in all probability the post-cathodal depression (Erlanger and Blair, 1931).

Monnier and Bacq (1935), in discussing Wedensky inhibition made the statement that only the electrical theory of neuromuscular transmission can account for all the aspects of the phenomenon. It is therefore of importance to emphasize that the argument developed in this section accounts satisfactorily for the experimental facts considered without any necessity of postulating an electrical transmission.

*C. Curare.* The Wedensky inhibition obtained after curare resembles that of fatigued muscles in that acetylcholine is disinhibitory. According to the explanation proposed above this inhibition would therefore result from the rapid decline of the quanta to the subthreshold levels. There is no evidence, however, that curare decreases the amounts of acetylcholine released by the nerve impulses; indeed, Dale, Feldberg and Vogt (1936), on stimulation of the motor nerves of fully curarized muscles, obtained a normal release of acetylcholine into the perfusing fluids. The effects of curare are readily explained if it is assumed that the drug raises both the threshold level and the upper paralytic boundary of the effective range so that larger amounts of acetylcholine are necessary for sustained contractions (cf. Dale, Feldberg and Vogt, 1936; Briscoe, 1936c). With such an assumption the antagonistic effects of eserine and curare (fig. 13) are easily understood.

The necessity for repetitive stimulation when single volleys are ineffective in the second stage of curarization is also reasonably accounted for; notwithstanding the decline of the quanta on repetitive stimulation, if the frequency is sufficiently high, enough acetylcholine accumulates at the beginning of stimulation so that the effective concentrations are reached.

The explanation adopted is meant to apply exclusively, for the present, to the effects of curare. The term "curarization" has been extended at Lapicque's (1926) suggestion to cover all instances in which motor nerve impulses fail to elicit responses while the muscles contract on direct stimulation. It is possible, however, that different drugs might lead to such a condition by different mechanisms. Feng (1936) has suggested that the mechanism must be the same in all cases, for in all cases calcium salts have a restoring action. Since both curare and eserine are listed among the "curarizing" substances studied by Feng, and since these two substances have opposite effects according to the evidence presented here, Feng's argument does not appear to be conclusive.

*D. Fatigue.* The theory developed in section E leads to the conclusion that fatigue of the neuromuscular junction denotes a decrease of the quanta of acetylcholine liberated by the motor nerve impulses to subthreshold levels. The progressive decline obtained experimentally is accountable by the fact that the responses are the sum of the contractions of many muscle fibers. If, as stimulation is repeated, the quanta of acetylcholine fall below the effective range in more and more of the junctions, the contractions of the muscle as a whole will gradually become smaller. For a single muscle fiber the theory implies, however, that contractions should cease suddenly, not gradually. Future studies will test the accuracy of this implication.

The probable variability of the thresholds and the span of the effective range for the different fibers of the muscle furnishes an explanation of the complex effects of acetylcholine—both stimulant and depressant—at certain stages of the muscular responses to tetanic stimulation (pp. 241, 245). It is plausible that injected acetylcholine raises the concentration at some fibers from the subthreshold to the effective level, thus inducing contraction, while it also raises the concentration of others from the effective to the paralytic level.

Indirect evidence in support of the assumption that repetitive stimulation leads to a reduction in the amounts of acetylcholine liberated per nerve impulse is provided by the observation of Bouman (1932) that in fatigue, as during curarization (p. 246), a stage occurs in which single volleys fail to activate while tetani still elicit contractions. If fatigue had reduced the quanta of acetylcholine below threshold, as the assumption states, tetanic stimulation could accumulate the mediator so that threshold would be reached. Direct evidence for the assumption has been furnished by Dale, Feldberg and Vogt (1936), who failed to detect a release of acetyl-

choline in fatigued muscles. Similarly, Brown and Feldberg (1936b) have shown a progressive decrease of the output of acetylcholine by the superior cervical sympathetic ganglion on prolonged repetitive stimulation of the preganglionic fibers.

In a previous paper (Rosenblueth, Lindsley and Morison, 1936) the conclusion was drawn that fatigue and curarization differed fundamentally, because eserine and acetylcholine depressed the responses of fatigued muscles, whereas they augmented those of curarized preparations. The present data (sections I and III) show that the basis for that conclusion should be qualified, since eserine and acetylcholine may also augment the responses of fatigued muscles. The distinction between fatigue and curarization appears now to be the following: in both conditions the concentrations of acetylcholine liberated by the nerve impulses are subthreshold; but in fatigue it is actually the quanta which are decreased, and in curarization normal quanta fail because of a heightened threshold.

E. *The post-tetanic increments.* It was pointed out in section A that the duration of the post-tetanic effects (up to several minutes) is such as to exclude the possibility that acetylcholine might be responsible for them. It was also pointed out (p. 249) that a mobilization of potassium ions might account for these effects. In support of this view is the observation of Wilson and Wright (1936) that injections of potassium salts result in an increment of the twitches of normal muscles.

After eserine the post-tetanic effect may be a depression, instead of an augmentation (fig. 12B). This effect is not in contradiction with the hypothesis tentatively adopted, for Wilson and Wright also reported depressant effects of large doses of potassium. Similarly in the superior cervical ganglion small doses of potassium are stimulant, but large doses have a paralytic action (Brown and Feldberg, 1936a).

The electrical responses of the muscle during the post-tetanic increments are probably repetitive (fig. 7). Yet the possibility of a concomitant contracture is not excluded. Frequently the height of the twitches is not altered after a tetanus of a fresh muscle, whereas the duration of the twitches becomes markedly greater. Such a change argues in favor of a contracture—repetitive firing of the muscle should lead to greater tension. Obviously more data are necessary before speculation may be replaced by theory in this regard. At this stage, however, the assumption appears reasonable that all the post-tetanic effects, including the decurarization (p. 246), may be caused by a single substance, liberated or mobilized by the acetylcholine set free by the nerve impulses. It is pertinent to recall that potassium has a decurarizing action (Wilson and Wright, 1936).

Besides the influence of a tetanus on the responses to single shocks, thus far discussed, another post-tetanic action was reported—the contraction which succeeds a brief tetanus after eserine (fig. 10). Prolonged stimulation is not succeeded by such a contraction. The tension develops at

least partly as the result of conducted disturbances in the muscle (p. 245). This action appears of theoretical importance as follows. Cowan (1936b) has argued that the simple statement that high concentrations of acetylcholine produce paralysis is misleading in so far as it ignores the later behavior of the system. If a high concentration, leading to Wedensky inhibition, has been attained after rapid nerve stimulation, should not the muscle contract again as this concentration subsides by destruction of the acetylcholine? It is possible that the post-tetanic action under discussion reveals precisely the possibility of answering this question in the affirmative. An attempt to account for the absence of this phenomenon after prolonged stimulation would be at present purely speculative.

#### SUMMARY

The effects of eserine, curare, acetylcholine and potassium chloride were studied in relation to the mechanical and electric responses of cat's skeletal muscle to indirect stimulation at various frequencies.

Falls of tension in normal muscles stimulated tetanically can be accounted for by some fibers becoming idle, although the nerve volleys are maximal (fig. 2). Repetitive discharges from a single nerve volley may occur during a tetanus (fig. 1). Wedensky inhibition occurs in unfatigued muscles at high frequencies of stimulation (fig. 4).

Eserine, acetylcholine and potassium can either increase or decrease the muscular responses (figs. 3, 5, 8, 9, and 11). The rate of stimulation and the fatigued or unfatigued condition of the muscle determine the appearance of either of the opposite effects of the drugs.

Eserine and curare favor Wedensky inhibition at low frequencies (figs. 2 and 6). Their effects do not sum, but cancel. Acetylcholine increases the depression of eserinizated muscles, whereas it disinhibits curarized muscles.

Post-tetanic increments of the responses to single maximal volleys occur in normal (figs. 4 and 7), eserinizated (fig. 12A) and curarized (p. 246) muscles. After eserine a post-tetanic depression may be observed (fig. 12B).

With the exception of the post-tetanic effects all the previous and present results are explained by the hypothesis that acetylcholine is the chemical mediator of motor nerve impulses (p. 247). Both a superabundance and a deficiency of acetylcholine may lead to depression of the muscular responses (fig. 14).

Fatigue is explained as due to a decline of the quanta of acetylcholine released by the nerve impulses so that the concentration becomes sub-threshold. Curare produces a similar condition by raising the threshold of the fibers, although the amounts of acetylcholine released are normal (p. 252).

Paralysis because of superabundance of acetylcholine may be produced in normal muscles if a high frequency of indirect stimulation is used (p.

252). Relatively low frequencies will elicit a similar condition after eserine (p. 251). Wedensky inhibition of this type differs, therefore, from that of fatigued or curarized muscles.

The theory of electrical transmission fails to account for the data (p. 248); its postulation as a subsidiary mode of transmission is unnecessary (p. 252).

The post-tetanic effects cannot be accounted for by either the electrical or the acetylcholine theory of transmission (p. 254). They lead to the conclusion that the nerve impulses induce other more enduring reversible chemical changes.

#### REFERENCES

- BOUMAN, H. D. *Arch. Néerlan. Physiol.* **17**: 307, 1932.  
 BOYD, T. E. *This Journal* **100**: 569, 1932.  
 BREMER, F. AND J. TITECA. *Arch. Internat. Physiol.* **42**: 223, 1935.  
 BRISCOE, G. *The Lancet* **1**: 469, 1936a.  
     *J. Physiol.* **86**: 1P, 1936b.  
     *Ibid.* **87**: 425, 1936c.  
 BROWN, G. L., H. H. DALE AND W. FELDBERG. *Ibid.* **87**: 394, 1936.  
 BROWN, G. L. AND W. FELDBERG. *Ibid.* **86**: 290, 1936a.  
     *Ibid.* **88**: 265, 1936b.  
 CANNON, W. B. AND A. ROSENBLUETH. *This Journal* **119**: 221, 1937.  
 COWAN, S. L. *J. Physiol.* **86**: 61P, 1936a.  
     *Ibid.* **87**: 43P, 1936b.  
 DALE, H. H., W. FELDBERG AND M. VOGT. *Ibid.* **86**: 353, 1936.  
 DAVIS, H. AND P. A. DAVIS. *This Journal* **101**: 339, 1932.  
 EASSON, L. H. AND E. STEDMAN. *Proc. Roy. Soc. B* **121**: 142, 1936.  
 ECCLES, J. C. *Ergebn. d. Physiol.* **38**: 339, 1936.  
 ERLANGER, J. AND E. A. BLAIR. *This Journal* **99**: 108, 1931.  
 FENG, T. P. *Chinese J. Physiol.* **10**: 513, 1936.  
 GASSER, H. S. *Physiol. Rev.* **10**: 35, 1930.  
 GUTTMAN, S. A., R. G. HORTON AND D. T. WILBER. *Proc. Soc. Exper. Biol. Med.* **34**: 219, 1936.  
 HOFMANN, F. B. *Pflüger's Arch.* **93**: 186, 1903a.  
     *Ibid.* **95**: 484, 1903b.  
     *Ibid.* **103**: 291, 1904.  
 KATO, G., T. HAYASHI, T. OTA, M. NAKAYAMA, H. TAMURA, M. TAKENCHI, K. KANAI AND S. MATSUYAMA. *This Journal* **89**: 692, 1929.  
 LANGLEY, J. N. AND T. KATO. *J. Physiol.* **49**: 410, 1915.  
 LAPICQUE, L. *L'excitabilité en fonction du temps*. Paris, 1926.  
     *Compt. Rend. Soc. Biol.* **122**: 990, 1936.  
 LUCAS, K. *J. Physiol.* **43**: 46, 1911.  
 MONNIER, A. M. AND Z. M. BACQ. *Arch. Internat. Physiol.* **40**: 485, 1935.  
 PÁL, J. *Centralbl. f. Physiol.* **10**: 18, 1900.  
 ROSENBLUETH, A., D. B. LINDSLEY AND R. S. MORISON. *This Journal* **115**: 53, 1936.  
 SCHIFF, J. M. *Muskel- und Nervenphysiologie*. Lehr, p. 189, 1858.  
 WEDENSKY, N. *Arch. de Physiol.* **23**: 687, 1891.  
 WILSON, A. T. AND S. WRIGHT. *Quart. J. Exper. Physiol.* **26**: 123, 1936.

PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY  
FORTY-NINTH ANNUAL MEETING

Memphis, Tenn., April 21, 22, 23, 24, 1937

*The pathway taken by the impulse in the mammalian ventricles.* D. I. ABRAMSON and K. JOCHIM (introduced by L. N. Katz). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, and the Department of Physiology and Pharmacology, Long Island College of Medicine, Brooklyn, N. Y.

According to the widely accepted view of Lewis, the normal impulse spreads in a definite pattern on the ventricular surface, showing a difference of about 30 sigma between the first and last activated spots. This has been attributed to the faster spread through the subendocardial Purkinje arborization than through the ventricular musculature. However, the results were explained by De Boer as due to the temperature gradient between the interior and surface of the exposed heart. Several workers were unable to confirm Lewis' observations (Clement, Erfmann and H. Wiggers).

Recently, Robb has suggested that the spread through the ventricular wall is parallel to the spiral muscle bundles. At variance with Lewis' and Robb's conceptions is the anatomical finding of Abramson and his associates that a Purkinje network exists within the myocardium.

The spread of the impulse was reinvestigated in anaesthetized dogs using unipolar leads. The surface of the heart was kept moist and at body temperature. Under these conditions, the impulse arrived at all epicardial points within a few sigma. A region on the right ventricle near the middle of the septum was activated earliest, and the right ventricle generally preceded the left. Even at low surface temperatures, the time differences were less than those observed by Lewis.

Induced ectopic ventricular impulses were found to spread at equal rates in all directions on the heart's surface except for a lag across the septum. Cuts made along and across the muscle fibers were without significant effect on the conduction time. Cutting the right bundle branch did not significantly delay the arrival in the right ventricle of an ectopic impulse induced in the left.

These results are *not* in accord with Lewis' or Robb's views. They indicate that the impulse spreads from endocardium to epicardium at approximately the same rate as along the endocardium. They substantiate the view that the impulse travels over the Purkinje network within the myocardium as well as that beneath the endocardium and suggest that the conduction rate in the Purkinje fibers is faster than that in ordinary ventricular muscle fibers.

*Distribution of acoustic pathways in the medial geniculate bodies.* H. W. ADES (by invitation), F. A. METTLER (by invitation) and E. A. CULLER. University of Illinois, Urbana, and University of Georgia Medical School, Augusta.

Limens of auditory acuity for pure tones are obtained in cats by the conditioned-response technique. Tests are made on the seven frequencies representing the octaves from 125 cycles to 8000 cycles. By means of the Horsley-Clarke stereotaxic instrument, localized electrolytic lesions are made bilaterally (symmetrically) in the medial geniculate bodies. Modifications of the usual technique of the Horsley-Clarke instrument are necessary. First, the ear-plugs which fix the instrument in the inter-aural line must be short enough to avoid puncture of the tympanum, and yet sufficiently long to insure firm anchorage in the bony meatus. Secondly, incision of the concha, preliminary to the insertion of the ear-plugs, must heal thoroughly without infection in order to insure postoperative testing conditions consistent with preoperative conditions.

A unipolar tungsten wire electrode, insulated with enamel except at the tip which penetrates the brain, is connected to the negative terminal of a B-battery. A saline-soaked cotton plug in the cat's mouth, having sufficient area to insure burning only at the exposed tip of the tungsten electrode, is connected to the positive pole of the battery. Following recovery from the operation, the cats are again tested on the same frequencies to determine the nature and extent of hearing losses.

Complete destruction of one (left) medial geniculate body produced a loss of roughly 10 decibels over the entire range of test frequencies. Incomplete destruction of one geniculate produced a differential loss of about the same magnitude at 1000 cycles with a much smaller loss at the other frequencies. Results obtained from cats in which localized bilateral lesions were effected gave larger losses on certain frequencies, while limens on the others remained unchanged. Preliminary results thus indicate the following points: 1, destruction of one medial geniculate body causes a general loss in acuity, comparable to that resulting from the destruction of one hemi-cortex or one cochlea; 2, localized bilateral lesions affect certain frequencies more than others, giving evidence that the several frequencies traverse the geniculates by different pathways. Precise location of the areas involved in specific lesions cannot yet be stated, pending complete histological examination.

*The comparative efficiency of oil and soap enemas in eliciting defecation in dogs.* HARRY F. ADLER (by invitation), E. L. BORKON (by invitation) and R. D. TEMPLETON. Departments of Physiology of the University of Chicago and Loyola University School of Medicine, Chicago, Ill.

Borkon and Templeton (Am. J. Physiol., in press) reported that in dogs oil enemas elicit defecation sixty to seventy-five per cent of the times injected, while soap enemas are about ninety per cent effective. Their observations were complicated by a variable quantity of material in the colon (table 1).

To determine the efficiency of enemas independent of the quantity of other material in the colon our own experiments were performed thirty minutes after normal defecation. Stimulation of the anal sphincter by the enema tube was avoided by giving the enema through a cecostomy prepared several months previously.

First the dogs were allowed outside for fifteen minutes and only those animals which defecated were used in this series. Thirty minutes after defecation the animals were divided into three groups. In the control group a tube was inserted into the cecostomy and left for five minutes.

After removal of the tube the animals were allowed freedom for thirty minutes in quarters where they were accustomed to defecate. In the two remaining groups each animal received an enema of either 100 cc. of white mineral oil or 100 cc. of a one and one-quarter per cent green soap solution. Five minutes were allowed for enema injections. The tube was then removed and the dogs allowed freedom in the same quarters as the controls. To prevent undue irritation to the gut enema days were followed by either rest or control days.

In the control group, defecation occurred in only two per cent of the experiments. In the oil enema group, defecation occurred in 36 per cent of the experiments, while in the group receiving soap enemas defecation occurred in 60 per cent of the experiments.

TABLE 1

	TIME OF LAST DEFECTION UNKNOWN			30 MINUTES AFTER LAST DEFECTION		
	Number of experiments	Number of defecations	Per cent defecations	Number of experiments	Number of defecations	Per cent defecations
Control.....	54	20	31	135	3	2
Oil.....	55	32	58	81	29	36
Soap.....	31	27	87	75	45	60

*A mechanism of lymphatic absorption with special reference to serous cavities.*

LANE ALLEN and ELKIN VOGT (introduced by W. F. Hamilton). Departments of Anatomy and of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

Lymphatic absorption from the major serous cavities of vertebrates has been studied by means of direct observation of the absorption of suspension. A method is described whereby diaphragmatic lymphatics can be alternately injected and flushed of particulate matter, thus permitting the study of absorption under different experimental conditions. A method is also described whereby lymphatic absorption can be observed and studied in excised preparations and whereby von Recklinghausen's direct observations on lymphatic absorption may be repeated.

The filling of terminal subserous lymphatics is coincident with relaxation and stretching of tissues in which the lymphatics are situated, and lymphatic flow is the result of compression of surrounding tissue. The increase in lymphatic volume which accompanies relaxation of the diaphragm results in a relative negative pressure effective in "sucking" suspensions through the peritoneum and into the lymphatic lumen. Lymphatic endothelium and the overlying peritoneum are physiologically as well as anatomically "sieve-like" under the conditions necessary for absorption.

Studies of the lymphatic pumps of various serous cavities show many adaptations of structure to function. The mechanism of lymphatic absorption, however, was found to be essentially the same for peritoneal, pleural, pericardial cavities, and the pleuro-peritoneal cavities of lower forms.

Observations on the lymphatics of the web of the frog indicate that similar mechanism is operative for the lymphatics of tissues in general.

From direct measurements on rate of lymphatic absorption and the volume of efferent lymphatics, it was calculated that a rabbit may absorb

at such a rate as to remove from its peritoneal cavity a volume of fluid in excess of  $\frac{1}{2}$  its body weight in twenty-four hours.

*Lymphatic absorption in excised tissues.* LANE ALLEN and ELKIN VOGT (introduced by W. F. Hamilton). Departments of Anatomy and Physiology and Pharmacology, University of Georgia School of Medicine, Augusta. (Demonstration.)

The mechanism of lymphatic absorption and flow can be demonstrated by manipulation of the excised diaphragm. The absorption of ink placed upon the peritoneum of the diaphragm can be effected by stretching the tissue. Lymphatic volume increase which results in absorption can be observed without the aid of magnification. The flow of absorbed material can be effected by 1, subsequent manipulation of the diaphragm of the rabbit and by 2, simulating the pressure of the liver against the diaphragm of the dog.

Fixed preparations demonstrate the location and structural modifications of the lymphatic pumps of various serous cavities.

*Marked thyroid hyperplasia without change in respiratory metabolism by the treatment of hypophysectomized rats with thyreotropic hormone.* EVELYN M. ANDERSON and HERBERT M. EVANS. Division of Medicine and Institute of Experimental Biology, University of California, Berkeley.

The injection of thyreotropic hormone into hypophysectomized rats on a high iodine diet produced hyperplasia of the thyroid without raising the oxygen consumption of 34 per cent minus. KI (10-100 gamma doses) given with thyreotropic hormone to guinea pigs inhibited the characteristic rise in oxygen consumption without preventing hyperplasia of the thyroid.

*A new smooth-muscle bath.* F. F. ANDERSON (introduced by A. R. McIntyre). The University of Nebraska Medical College, Omaha. (Demonstration.)

It is possible with this bath to change completely the solution in which the preparation is suspended without exposing the preparation to the air and without any disturbance of the record by the flow of the fluid. The incoming fluid is not exposed to temperatures outside the water-bath and the side limbs are so constructed that their capacities are approximately 1 ML only, consequently errors caused by diffusion are minimal. Washings are controlled by a single stopcock so constructed that it will operate if necessary without grease. Changes of the bath-contents can be made so that the volumes are automatically of equal volume. Provision is made for the passage of gas through the incoming solution so that correct  $\text{CO}_2$  or  $\text{CO}_2\text{--O}_2$  tension may be maintained. The bath is simple in operation and very convenient in use.

*The effects of fatigue due to muscular exercise on the Purkinje cells of the mouse at various ages.* WARREN ANDREW (introduced by W. F. Hamilton). Department of Anatomy, School of Medicine, University of Georgia, Augusta.

A study has been made of the effects of fatigue on the Purkinje cells of mice at ages ranging from a time when the animals are first capable of prolonged running until they have reached extreme senility. Exercise

has consisted of running in a motor-driven rotary cage built for the experiment. Control animals of the same brood have been killed and sections of their cerebella stained with methylene blue in the same slide-tray with those of the fatigued animals. Purkinje cells in corresponding parts of the cerebella have been compared as to cell volume, nuclear volume, nucleoplasmic ratio, amount and distribution of Nissl substance, basophilia of nucleus, and some other items.

Definite, but not striking, differences are seen in Purkinje cells of fatigued animals when compared to those of controls. The changes consist chiefly in an increased staining capacity of the nucleus, a loss of Nissl substance, or hypochromatism of the cytoplasm, and changes in volume of nucleus and cell-body.

Changes in the Purkinje cells of senile rodents, previously studied by the author (Andrew, 1936) are far more marked than are changes due to fatigue from muscular exercise. These changes include increased staining capacity of the nucleus, hypochromatism of the cell-body, and a binucleate condition in a number of the cells. The presence of more than one nucleus in many Purkinje cells in senile mice substantiates the findings of Tetsuo Inukai in senile rats and is a phenomenon which we have seen in no cases except those of very old animals. Some of the differences between Purkinje cells of fatigued and senile animals may be differences of degree only, others are apparently qualitative differences.

*A slow parasystolic focus with exit-blocking.* RICHARD ASHMAN and JAMES L. GOUAUX (by invitation). Department of Physiology, Louisiana State University Medical Center, New Orleans.

From a healthy boy, aged 9, three series of electrocardiograms, recording over 2000 heart cycles, were taken at intervals of about a month. Ventricular premature beats appeared frequently at any phase of diastole excepting very early, and clearly emerged from a rhythmic ectopic focus. The focal rate varied from a mean of about 65 when the sinus rate was about 105, to 53 or less when the sinus rate was 65 or 70. The focal rate fluctuated with respiration, though only one-fourth as much as the sinus rhythm.

Exit-blocking of focal impulses was common when the sinus rate was rapid, rare when slow. Of 228 focal impulses on one occasion when the sinus was rapid, 108 were manifest and 120, blocked. Every phase of the heart cycle was adequately tested. Of 29 impulses due to emerge from the focus at 0.33 to 0.36 sec. after the R wave, 52 per cent were successful, while many of the failures were due to ventricular refractoriness. From 0.36 sec. percentage success declined until, at between 0.41 and 0.46 sec. after R, only 3 (6 per cent) of 49 impulses caused premature beats. From 0.46 to 0.55 sec. percentage success rose rapidly to 100, where it remained throughout diastole.

On the other dates the same phenomenon was seen, though exit-blocking was infrequent because the heart rate was slower.

The factors involved in the exit-blocking are related to 1, nervous influences; 2, refractoriness; 3, and, possibly, production of metabolites. These are receiving further study.

We believe this case throws light on the interpretation of certain phenomena seen in A-V heart-block and attributed to a supernormal phase.

*The respiratory quotient and carbohydrate utilization as affected by exercise taken immediately and thirty minutes after the ingestion of glucose and of fructose.* GEORGE BACHMANN, JOHN HALDI, W. WYNN (by invitation) and C. ENSOR (by invitation). Laboratory of Physiology, Emory University School of Medicine, Atlanta, Ga.

Two series of experiments were conducted on each of two male adults. After the determination of the basal metabolism the subjects, in one series, rode a bicycle ergometer for 30 minutes immediately after the ingestion of 200 cc. water, 20 grams glucose or 20 grams fructose dissolved in 200 cc. water, the experiments with water serving as controls; in the other series, they rode for the same length of time but began the exercise one half hour after the ingestion of water or the sugar solutions. Work was done at a constant rate of 550 kilogrammeters per minute. Exercise when taken immediately after ingestion will be referred to as "immediate" and when begun 30 minutes later as "delayed."

Since practically the same results were obtained on each subject, only averages derived from the data on the two subjects will be given. In the control experiments with water the non-protein respiratory quotients were virtually the same during immediate and delayed exercise, being approximately 0.12 above the basal level in each instance. In the experiments with both glucose and fructose, the quotients during immediate exercise were not appreciably different from those in the control experiments, but were higher during delayed exercise, the increase above the basal with glucose being larger than with fructose. In the experiments with glucose the average net increase of the quotients of delayed exercise was 0.04 higher than that of immediate exercise, and in the fructose experiments 0.03 higher. The net increase was obtained by subtracting the increase above the basal level in the control experiments from the increase above the basal in the sugar experiments. The net increase in grams of carbohydrate oxidized in the glucose experiments was 6.0 grams more during delayed than during immediate exercise, and in the fructose experiments 4.3 grams more.

It is therefore concluded that the amount of carbohydrate oxidized during exercise was appreciably increased by the ingestion of the sugars 30 minutes before beginning the exercise, and to a greater extent with glucose than with fructose, whereas it was unaffected when the same amount of these sugars was ingested immediately before exercise.

*Rôle of the upper gastrointestinal tract in the etiology of pernicious anemia. An experimental study in dogs.* WILLIAM H. BACHRACH and SAMUEL J. FOGELSON (introduced by C. A. Dragstedt). Departments of Physiology and Experimental Surgery, Northwestern University Medical School, Chicago, Ill.

Following the experiments of Castle in 1928 which demonstrated the absence of a specific factor in the gastric juice of pernicious anemia patients, numerous attempts have been made to localize the site of elaboration of the so-called intrinsic factor. Meulengracht (1935) and Ungley (1936) found that the active principle was present in the pylorus but not in the fundus, and Sharp (1931) and Meulengracht (1935) showed that the duodenum was also active. The histologic similarity of Brunner's glands to the pyloric glands led Meulengracht to suggest that these glands may be the site in the upper gastrointestinal tract where the antianemic

factors are formed. This hypothesis has been advanced to explain why, after gastrectomy, pernicious anemia occurs infrequently in man and never experimentally. Petri (1936) and his co-workers were unable to produce the disease in dogs by removal of the stomach and the Brunners gland-containing portion of the duodenum.

The present studies were made on a group of dogs in which all of the pyloric and Brunners glands were removed with the additional resection of sufficient intestine to remove the probable margin of safety. The operation consisted of excision of the distal three-fourths of the stomach, all of the duodenum, and the first 30 cm. of the jejunum, the bile and pancreatic ducts being transplanted into the jejunum.

Seven dogs have been observed for more than one year. Following the post-operative anemia, the blood picture has slowly returned toward normal. Four of the animals have been achlorhydric to alcohol test meal throughout the observation period.

To date, none of these dogs have shown any of the hematologic, neurologic, or gastrointestinal manifestations of pernicious anemia.

*A direct current thermostromuhr.* E. J. BALDES and J. F. HERRICK. Mayo Foundation, Rochester, Minn. (Demonstration.)

The platinum electrodes in the modified Rein diathermy-thermoelement which conduct the high frequency current to the blood vessel and blood are replaced by a specially designed direct current heater. This heater may consist of an insulated loop of rolled nichrome wire no. 38 B & S gauge which has a resistance of about 1.5 ohms per cm. length. Adequate heating without apparent injury to the blood vessel is furnished by a current varying from 200 to 300 milliamperes. For satisfactory operation of the Rein thermostromuhr about 20 milliamperes of high frequency current are required. Assuming a resistance of 400 ohms for blood vessel and blood, the heat dissipated amounts to 0.04 calorie per second. For corresponding sensitivity the d. c. thermostromuhr requires less heating energy.

This thermostromuhr differs appreciably in construction from that demonstrated last year by Schmidt and Walker. One difference lies in the fact that in their type of unit the thermocouple circuit requires the insertion of a potentiometric device to balance part of the thermal e.m.f. instead of being connected directly to the galvanometer. One point in common is that both types of unit are operated on direct current.

Calibration charts giving a comparison with the high frequency type of unit will be shown. Photographic records will indicate the time required for equilibrium during the measurement of blood flow. Several models of thermostromuhr units will be displayed.

*Analysis of the Rein thermostromuhr method of measuring blood flow.* E. J. BALDES and J. F. HERRICK. Mayo Foundation, Rochester, Minn.

The ingenious method of Rein for measuring blood flow is probably the most accurate that has been devised for general study in physiologic preparations. With the use of aseptic surgical technic and a thermostromuhr unit that can be sterilized, the measurement of blood flow in the intact animal is made possible.

An investigation of the *modus operandi* of the Rein thermostromuhr leads to a contradiction of the fundamental concepts given by Rein in

explaining the theory of the method. The assumption that the heat dissipated in the wall of the blood vessel by the high frequency current can be disregarded is not compatible with experimental evidence based on measurement of the relative high frequency resistance of blood and blood vessel wall. Also, the fact that the galvanometer always records a temperature difference along the wall of the blood vessel many times greater than can exist in uniformly heated blood fails to lend support to the theory.

Since the calibration of the thermostromuhr and the measurement of blood flow is done empirically, the accuracy and reliability of this method is somewhat independent of the underlying theory. However, certain precautions immediately became apparent in the development of a successful technic. The chief of these is the discrimination between turbulent and stream line flow in the vessel under observation.

The analysis of the method leads to one fundamental question, namely, what is the difference between heat dissipated in the wall of the blood vessel and in the blood by heating with high frequency current and heat supplied externally to the blood vessel? In either case the temperature gradient along the wall is similar and dependent on the flow of blood inside the vessel.

Thermostromuhr units in which a direct current heater replaces the electrodes of the Rein unit have been built. It is believed that the new units for determining blood flow will allow measurements to be made more accurately and with greater facility. This type of direct current thermostromuhr seems to lend itself to calibration on blood vessels perfused with blood.

*The laws determining the relation of isometric tension to length in skeletal muscle.* M. GARCIA BANUS and A. ZETLIN (by invitation). Department of Physiology, Tufts College Medical School, Boston, Mass. (Read by title.)

Earlier investigations have indicated that the relation of isometrically developed tension to length in skeletal muscle obeys the following rules: the initial tension follows a hyperbola-like curve; the total tension follows Blix's S curve, while the developed tension follows a curve with a maximum.

Employing an isometric myograph of the non-friction, reflected beam type (amplification up to 500), and stimulating, with maximal single stimuli, the motor nerve of the gastrocnemius, sartorius, and rectus muscles of decerebrated cats or frogs with their circulation intact, the following results were obtained. The initial tension is an exponential function of the length expressed by the formula  $t = e^{lk} + a$ . The total tension curve consists of three parts: I, a straight line function of the length up to about 80 per cent of the maximum physiological stretch of the muscle in the body; II, a straight line plateau parallel to the length axis and extending usually beyond the maximum physiological length; III, a rapidly rising curve. This last part occurring sometimes at lesser stretches may encroach on, or partly obliterate the plateau. This whole curve may be changed to the S form of Blix in fatigued muscles.

Part III of the curve can be interpreted as an artefact due to the addition, to the tension developed by the muscle, of the very high initial tension developed by the connective tissue at unphysiological stretches, because: 1, it appears at lower stretches in muscles where the constant  $k$

(expressing the steepness of the initial tension curve) is greater; 2, it can be delayed by removing some of the connective tissue surrounding the muscle fibers, thus prolonging the plateau; 3, the effect at greater stretches is increased in an exactly additive way if the initial tension is increased by addition of connective tissue of a similar muscle.

In view of these facts, the following laws are tentatively suggested: the initial tension is an exponential function of the length; the total tension is a straight line function to a maximum, then remains constant; the developed tension is a mathematical consequence of the above two laws.

*Sensori-motor cortex and thalamus opticus.* J. G. DUSSEY DE BARENNE. Laboratory of Neurophysiology, Yale University School of Medicine, New Haven, Conn.

The sensory or sensori-motor cortex of the monkey occupies a large region of the pre- and postcentral cortex and is subdivided into three major subdivisions, a leg-, an arm-, and a face area (Dusser de Barenne, 1924). Local strychninization of a minute area of the cortex of any of these subdivisions, either in their precentral or postcentral portion, induces a marked hyperactivity of the corresponding thalamic nucleus or nuclei, evidenced by typical changes in the electrothalamogram.

This finding demonstrates a close functional interdependence of the sensori-motor cortex and the optic thalamus, for which the circuit formed by thalamo-cortical and cortico-thalamic neurones is the anatomical substratum.

*Prolonged fasting in the normal and depancreatized dog.* S. B. BARKER (by invitation), J. P. CHANDLER (by invitation) and WILLIAM H. CHAMBERS. Department of Physiology, Cornell University Medical College, New York City.

When one group of dogs was fasted until the loss in body weight reached about 50 per cent, the premortal elevation in endogenous protein catabolism was obtained. Associated phenomena were a rise in basal R.Q. to about 0.82, a fall, or even a complete disappearance of acetone bodies in the urine, and an increased creatinuria, often reaching as much as 20 mgm. per hour. After the ingestion of 50 grams of glucose by the animal in this condition, the R.Q. rose to 0.90, the high nitrogen excretion decreased, and about 2 grams of sugar were recovered in the urine. These immediate responses, indicating oxidation of the glucose, stand in contrast to the temporary inability to burn sugar shown by dogs fasted for 3 weeks.

A similar return of glucose oxidizing function was shown by other animals which were fasted to a 50 per cent weight loss without exhibiting a significant rise in protein breakdown or in basal R.Q. The ketonuria also remained nearly as high as the level existing in the early part of the fast, about 5 mg. per hour. The principal point of resemblance to the first group of animals was the increase in creatine output, although this was somewhat smaller, rising from an early value of 3 mgm. per hour to about 10. It is possible that glucose derived from increased protein metabolism may have enabled the animals to handle exogenous glucose more efficiently, but this explanation cannot hold for those which showed no heightened nitrogen excretion.

We have found that the depancreatized dog, although unable to oxidize ingested carbohydrate in the early stages of fasting, apparently gained

this ability when 50 per cent of the body weight was lost. The premortal increased endogenous protein breakdown did not seem to be a necessary factor for glucose oxidation. As the animals approached the terminal condition, creatinurias as high as 20 mgm. per hour were encountered, showing a marked rise even over the already high diabetic excretion of 3 to 10 mgm. per hour. These results indicate that the depancreatized dog reacts to prolonged fasting in the same manner as the normal dog.

*The effects of lesions of various parts of the vestibular system in macacus rhesus.* S. E. BARRERA and A. FERRARO (introduced by F. H. Pike).

Department of Neuropathology of the New York State Psychiatric Institute and Hospital, New York City.

In seventy macacus rhesus monkeys various lesions of the vestibular system were made, including labyrinthectomy, section of the eighth nerve, large unilateral vestibular nuclear lesion, lesions of the various nuclear groups, including the descending root and nucleus, the nucleus triangularis, Deiter's nucleus, and nucleus of Bechterew. In addition the connections of the vestibular nuclear complex with the cerebellar nuclei, via the juxtarestiform fibers, and the connections of the vestibular nuclear complex with other brain stem nuclei via the posterior longitudinal bundle were cut. A study was made in acute and chronic stages of the various characteristic phenomena associated with vestibular lesions. Many previous investigations have established these characteristic phenomena of vestibular dysfunction in experimental animals of various species in both the early and late post-operative stages. They include in acute stages postural asymmetries of the limbs, trunk and head, disorders of walking and climbing, disorders of motion in relatively homogeneous media such as air or water, and abnormal eye movements (nystagmus). Varying degrees of ipsilateral hypotonia and diminution of tendon reflexes may occur. In chronic stages only slight asymmetries of posture and motion may persist in addition to abnormalities of eye movements as detected by vestibular function tests.

Our experiments demonstrate that various elements in this vestibular dysfunction symptom complex may be elicited by lesions of any of the structural components of the vestibular system (labyrinth, eighth nerve, various vestibular nuclei, vestibulo-cerebellar connections, and posterior longitudinal bundle). The specific characteristics and the individual variations in intensity and direction of some of the phenomena were found to be related to the particular vestibular structures or connections involved in the lesions. All of the lesions were controlled anatomically by serial sections of the brain stem prepared by Nissl, Weigert or Marchi techniques.

*The influence of cholecystectomy and herniorrhaphy on gastric motility in man.*

LOUIS E. BARRON (by invitation) and GEORGE M. CURTIS. Department of Surgery, Yale University and Department of Research Surgery, The Ohio State University, Columbus.

The effects of cholecystectomy and herniorrhaphy on gastric motility in man were studied by the balloon and kymograph method. Two patients had cholecystitis with cholelithiasis. Cholecystectomy was eventually accomplished under nitrous-oxide-ether anesthesia. Gastric motility tracings were obtained in the morning, fourteen hours after the

preceding meal. The usual duration of each experiment was five hours. Observations made on the patients with biliary tract disease during an adequate control period indicated an increase in the duration of periods of gastric motility and an increase in the amplitude of the contractions. It was possible to obtain kymographic tracings during attacks of biliary colic. These revealed continuous motility with occasional contractions. Daily observations were resumed four days subsequent to cholecystectomy, and were continued until the patients were discharged from the hospital. These studies indicated intense persistent gastric motility lasting for approximately two weeks. The contractions were of higher amplitude than those of the control period. Subsequently the motility returned to normal.

Gastric motility was studied in five patients with inguinal hernia. Herniorrhaphy was eventually accomplished on four patients under low spinal anesthesia. For the fifth nitrous-oxide anesthesia was used. The observations made during the control period revealed normal gastric motility. Studies were made immediately preceding herniorrhaphy to note any disturbances in motility possibly associated with emotional changes resulting from anticipation of the surgical procedure. These demonstrated numerous short periods of intense gastric motility. Kymographic tracings obtained during herniorrhaphy on those patients operated on under low spinal anesthesia revealed complete gastric inhibition. This was observed throughout the entire operation and persisted from twenty-four to seventy-two hours subsequently. Following this there was a gradual return of tonicity and contractility. Twenty-four hours after the onset of motility, hypermotility ensued. This was characterized by an increase in the duration of periods of gastric motility and an increase in the amplitude of the contractions. This persisted for approximately two weeks. Eventually the motility returned to normal.

*A study of the total acid soluble phosphorus in skeletal muscle of rats.* A. J. BARTOLI, JEANNETTE L. COHEN and H. C. STRUCK (introduced by C. I. Reed). Department of Physiology, College of Medicine, University of Illinois, Chicago.

Previous studies have shown (H. C. Struck and M. B. Visscher, *Proc.*, 1935) that, in the particular group of animals studied, the total acid soluble phosphorus of skeletal muscle in rats was decreased markedly in extreme old age. Most of this decrease was in the creatine phosphate and adenyl pyrophosphate fractions, a fact which is of interest in view of the lessened activity of senile animals.

Further study has shown that the total acid soluble phosphorus of skeletal muscle remains relatively constant during the first half of life, and that the decrease occurs after the rat has attained the age of approximately fifteen months.

A more extended investigation of the phosphorus content of muscle as affected by various dietary procedures has been carried to the age of sixteen months in an effort to see if the phosphorus content can be modified by the procedures studied. In addition to the normal diet, serving as controls, high phosphorus diet, excess vitamin B<sub>1</sub> and D, and growth hormone have been administered.

The results indicate that no significant changes in the total acid-soluble phosphorus of the gastrocnemius muscle occurs during the first ten months

of life under the various regimes tested; and that at the age of sixteen months, all groups had begun to show decreases in phosphorus content. While certain groups show statistically insignificant decreases, the trend is, nevertheless, clear; and it may be of interest that these dietary modifications tend to delay the onset of the decrease in phosphorus content.

*Poikilothermic changes in man and their effect on respiratory exchange.*

H. C. BAZETT, S. GOLDSCHMIDT, B. MCGLONE and L. SRIBYATTA<sup>1</sup> (by invitation). Department of Physiology, University of Pennsylvania, Philadelphia.

A fall of alveolar CO<sub>2</sub> tension and rise in R.Q. is recognized as an accompaniment of hyperthermia; it is commonly attributed to skin stimulation by heat. This explanation is improbable since the opposite effect of retention of CO<sub>2</sub> and decrease in R.Q. may accompany a rapid fall of rectal temperature, provided that this fails to induce a maintained reactive increase in metabolism. Such a fall of temperature may develop in prolonged cool baths (30° to 32°C.) in some subjects; it is more readily attained by following such a bath of  $\frac{1}{2}$  hour's duration with another about 1° warmer. With removal of skin stimulation by cold, vascular relaxation develops and the rectal temperature falls rapidly to levels of 35.6 to 36.0° in most subjects without shivering. Respiration is commonly depressed, shallow and irregular, and resembles in character that of a subject responding to oxygen lack. Alveolar CO<sub>2</sub> tension may be increased, oxygen tension lowered, and R.Q. values below 0.7 may be recorded. The reaction is not evident if the cooling is too gradual, nor if it is too rapid, for the metabolic changes associated with the onset of shivering are accompanied by a rise in R.Q. The retention of CO<sub>2</sub> is associated with the fall in internal temperature (though after some lag) and not with changes in mean body temperature, for it may develop in the early stages of warming when the body is accepting heat from the bath. This heat acceptance may be demonstrated by a study of thermal gradients in the skin, or by calorimetry, even when the internal temperature is falling. Presumably the effects described depend on acid-base adjustments necessitated by the effect of temperature changes on the buffer action of proteins and on the solubility of CO<sub>2</sub>.

*The mode and site of action of oxygen at increased barometric pressures on the mammalian organism.* JOHN W. BEAN and GERALD ROTTSCHAER (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Dogs were decerebrated under morphine-urethane, Evipal or temporary ether anesthesia. The cerebrum was removed down to the superior colliculi except for a few preparations in which the thalamus was left intact. The animals were exposed to commercial oxygen at from three to five atmospheres pressure. Blood pressure, heart rate and respiration were recorded and observations of the animal made.

The response to high oxygen in these preparations includes increased breathing and dyspnea, slowed heart rate, slightly or markedly increased blood pressure, and convulsive reactions typical of acute oxygen poisoning.

<sup>1</sup> Professor Sribyatta assisted in the origination of these experiments in 1928, when he held a Rockefeller Fellowship.

The occurrence of such reactions in the decerebrate animals localizes their sites of origin to structures below the cortex and the basal ganglia. If afferent impulses from the carotid sinuses or vagi are contributory to the oxygen response they are not essential to the production of the convulsive reaction. Denervation of the carotid sinuses alone, vagal sectioning or blockage alone, or vagal blockage after sinus denervation does not avert the convulsive movements. The respiratory and body movements obtaining as a result of exposure to high oxygen are found to be so strikingly similar to those obtaining during cold blockage of the vagi at atmospheric pressure as to suggest the high oxygen acts to paralyze the normal vagal influence over the respiratory mechanism.

The slowed pulse rate, the slowed deepened respiratory movements obtaining during exposure, the rapid reversal of these effects and the subsidence of convulsive movements when decompression is carried to just below three atmospheres or when high oxygen is replaced by room air at an equivalent barometric pressure, support the belief that a retention of carbon dioxide in the tissues due to a broken coordination of the dual function of the hemoglobin is an extremely significant factor in the production of the oxygen poisoning effects. The respiratory nature of the convulsive movements points to the central respiratory mechanism as the primary site of acute oxygen poisoning. That high oxygen may exert some direct toxic, central or peripheral action is not denied.

*On the properties of neuronin, the principal protein complex of neuroplasm.*

RICHARD S. BEAR (by invitation), FRANCIS O. SCHMITT and JOHN Z. YOUNG (by invitation). Departments of Zoology, Washington University, St. Louis, Mo., and Oxford University, England.

Axis cylinder material uncontaminated by other tissue components may be obtained from giant axons of the squid merely by cutting the ligated axons and extruding their contents. The nature of the protein of such extruded material has been investigated.

Except for a very small portion, neuroplasm is soluble either in water or salt solutions. Such solutions are similar in all respects thus far investigated to neutral extracts of other nervous tissues (crustacean peripheral nerve and dried lipoid-free mammalian central nervous system). For convenience and to avoid certain erroneous implications involved in previously proposed names for this protein, here shown to be characteristic of neuroplasm generally, we term this protein complex "neuronin."

Neuronin has the solubility properties of a nucleio- or pseudonucleoprotein. Under proper conditions a basic protein similar in solubility to a histone or protamine can be demonstrated after brief action of dilute alkali and removal of the main fraction by acid heat coagulation. A phosphorus-containing fraction, small in amount and having certain properties resembling those of animal nucleic acid, can be isolated after removal of the bulk of the protein by acid heat coagulation in the presence of high salt concentration. Definite characterization of the latter fraction is difficult because of its low proportion and because of the relatively small amount of squid material available.

Since neuronin is the principal protein of the axis cylinder its colloidal properties are of importance in the determination of the submicroscopic structure of the axon. Apparently the protein remains in a relatively dispersed condition in the living axon because of solvation due to dissolved

salts. That relatively small changes in the salt concentration or in pH may be capable of altering the state of aggregation of this labile system in the living axon seems probable.

*The effects of magnesium on the production of acetylcholine by motor nerve stimulation.* L. J. BELNIAK (by invitation) and HAROLD N. ETS. Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

Dale, Feldberg and Vogt have shown that acetylcholine is liberated at the endings of a stimulated motor nerve in the cat, and that curare does not prevent this effect. Using the perfused tongue of the dog, we have confirmed this finding.

Similar observations have been made on preparations "curarized" with  $MgSO_4$  (Lubinska, Arch. int. de Physiol. 41: 456, 1935; Brosnan and Boyd, Proc. Soc. Exper. Biol. and Med. 35: 405, 1936). The tongue was perfused with Locke's solution containing added eserine and sufficient  $MgSO_4$  to abolish all response to indirect stimuli (single or tetanic). Samples were collected before, during, and after a 4 to 6 minute period of tetanization of the hypoglossal nerve at a frequency of 100 per second, and were tested for acetylcholine activity. The assay was carried out on the frog rectus abdominis and leech muscles, and the results obtained by the two methods were comparable.

Responses of both these preparations are depressed by the presence of Mg. Satisfactory comparisons were made possible, however, by adding to the standards of known acetylcholine content the same concentration of Mg used in the perfusate. The Mg depression is quickly reversible in Mg-free solutions.

The initial resting samples showed no acetylcholine (except a trace in one experiment out of nine). The concentration is highest during and immediately after stimulation, progressively diminishing thereafter. So far as quantitative comparison is possible, the amount of acetylcholine recovered in the magnesium experiments seems at least as great as in the normal controls.

*Fright and drug contractions in denervated facial and ocular muscles.* M. B. BENDER (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Beginning at about eight days after sectioning the seventh or the third cranial nerve of monkeys slow spasmodic contractions of the denervated muscles occur whenever the animal becomes frightened. The contraction appears a few seconds after the initial period of fright, lasting about 3 seconds and disappearing within the next 5 to 7 seconds. If the monkey is startled repeatedly by the same method, i.e., a menacing gesture, the contraction fails to appear after several trials. New methods of frightening, however, serve to bring out the reactions. These slow contractions could not be reproduced by intramuscular injection of epinephrine or by stimulation of the cervical sympathetic trunk. Their occurrence during fright was not prevented by atropine. Eserine augmented the fright reaction in some, but not in all monkeys. Section of the cervical sympathetic on the side of the denervated facial muscles definitely intensified the contracture, particularly in *m. orbicularis palpebrarum* during fright and even at rest. Intramuscular injection of epinephrine in such a prepa-

ration abolished the intensifying effects of cervical sympathectomy. The similar slow contraction in the denervated muscles were reproduced in every case by intramuscular injection of eserine (0.05 mgm. per kilo) followed by injection of acetylcholine (0.8 mgm. per kilo). The drug contraction appeared from 1 to 2 minutes after the injection and lasted for 10 to 30 minutes. Injection of epinephrine at the height of the contraction abolished it within one to two minutes, for a period of 15 minutes. The heart rate in these instances was slow and the beat forceful. Frightening the monkey during the period of action of acetylcholine immediately decreased the contraction slightly, but after 1 to 3 seconds the contraction returned. Both fright reaction and the drug contraction decrease in intensity when regeneration of nerve function can be demonstrated.

From these observations it is inferred that the fright reaction in the denervated muscles mentioned is produced by cholinergic innervation from some source other than the cervical sympathetic.

*A synchronous motor electric time clock.* A. L. BENNETT (introduced by A. R. McIntyre). The University of Nebraska Medical College, Omaha. (Demonstration.)

This clock provides electrical contact at intervals of 1, 2, 5, 10, 15, 30 and 60 seconds as well as 5, 15, 30 and 60 minutes without adjustment of any clock contacts. Two switches provide simple and instantaneous setting for any of the intervals. A third switch makes it possible to superimpose a triple marking at 1 minute intervals when recording at any interval less than 1 minute. Contact is short and uniform regardless of interval. The clock is accurate, dependable and entirely adequate for timing research experiments or as a master clock for a general laboratory. Furthermore it may be easily made in a laboratory workshop at a very nominal cost. A small synchronous electric clock motor is mounted on a panel of insulation material. Contacts are inserted through the panel so that the "second" hand makes contact with the outer circle of sixty points while an extension of the "minute" hand reaches the inner circle of twelve points. All wiring is done on the back of the panel. The front of the dial is polished so that the contacts are flush with the surface and the hands slide smoothly over metal and insulation material alike. In order to use the 110-A.C. current to activate the relays as well as run the clock, a 10,000 ohm, sensitive relay is placed in circuit with the clock contacts. This draws only 2.5 milliamperes and thus protects the clock contacts from burning and becoming rough. A second relay, activated through the first, has heavy enough contacts to carry the load of several signals if the clock is to be used as a master timer for a large laboratory. Since there is no adjustment necessary at the clock contacts a glass cover may be fastened tightly over the dial, thus making it dust proof.

*The variability of the energy of metabolism in normal individuals.* JOSEPH BERKSON and WALTER M. BOOTHBY. Division of Biometry and Medical Statistics, and the Section of Clinical Metabolism, The Mayo Clinic, Rochester, Minn.

This report continues and amplifies previous studies published in this journal on the standardization of normal metabolism. The form and amount of the variability of metabolism of normal individuals measured from the standard or mean metabolism for individuals of the same sex and

age were studied and evaluated. This variation (total variability) can be thought of as made up of the variation of each individual's metabolism around his or her mean metabolism (intra-individual variability) and the variation of the mean metabolism of different individuals around the mean metabolism of all persons of the same sex and age (inter-individual variability). With the total variability evaluated, the inter-individual variability can be estimated by formula, if the intra-individual variability is known. To determine the intra-individual variability a special series of subjects were available for each of whom there were eight or more repeated determinations. Each individual's series was studied separately and all were combined to give a composite picture of the distribution of repeated determinations of the same individual. These results studied together with those for the total variability, enabled us to evaluate the inter-individual variability.

*Peripheral vasomotor effects of CO<sub>2</sub> locally applied.* THEODORE BERNTHAL and JOHN C. SHOEMAKER (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

The direct effects of variations in blood CO<sub>2</sub> tension upon blood vessel caliber in the foreleg were studied (dog; urethane-morphine). The index of vasomotor reaction was alteration of volume flow of blood through the perfused innervated or denervated foreleg while both the pulsatile arterial (perfusion) pressure and the venous (outflow) pressure were artificially maintained at non-varying mean levels. Inflow was measured by Gesell's and Bronk's thermoelectric method, and outflow, in some of the experiments, was measured by a simple "tipping bucket" method.

While the O<sub>2</sub> tension of the perfusing blood was carefully maintained at a constant level (approximately 105 mm. Hg), known alterations of CO<sub>2</sub> tension were produced for periods of from 1 to 3 minutes' duration. A basic level of approximately 43 mm. CO<sub>2</sub> tension was used in all determinations and the alterations from this level were downward to 3 or 4 mm. and upward to 65, 95 and 130 mm. Hg.

In most cases, increasing the CO<sub>2</sub> tension diminished the blood flow and decreasing the CO<sub>2</sub> tension increased the blood flow. Denervation of the leg sometimes accentuated the responses. In a small per cent of cases, the effects were indeterminate or in directions opposite to those stated above.

Thus, while the hypothesis of Fleisch and collaborators (Pflüger's Arch. **230**: 814, 1932) that, by direct action, CO<sub>2</sub> may exert simultaneously opposing constrictor and dilator effects upon arteries and capillaries respectively is neither confirmed nor denied, their conclusion that the dilator phase dominates the blood flow reaction is not supported by the present experiments upon the dog's limb which confirm those performed earlier with a different but less well controlled technique. (Am. J. Physiol. **95**: 446, 1930.) The unqualified statement that CO<sub>2</sub> causes vasodilatation by its direct action upon the peripheral vessels seems not to be justified.

*The administration of heparin.* C. H. BEST, ARTHUR CHARLES (by invitation) and CAMPBELL COWAN (by invitation). School of Hygiene, University of Toronto, Canada.

Solutions of crude heparin administered subcutaneously to dogs or to other laboratory animals, usually produce little or no effect upon the clot-

ting time of the blood. When very large doses are given to small animals a response may be observed but this is usually irregular and evanescent.

When solutions of highly purified heparin containing approximately 15,000 Howell units per cubic centimetre are administered by the subcutaneous route a prompt and prolonged effect upon the clotting time of the blood is uniformly secured. The effect of one cubic centimeter of this material on the clotting time of the blood of an anaesthetized dog persists for some twelve hours, i.e., two doses per day are sufficient to secure continuous heparinization of animals weighing from 12 to 15 kgm.

While the protamine, salmine, produces a precipitate when added under certain conditions to heparin solution, the nature of the complex is not such that it is readily suspended in water. Benzidine, however, precipitates heparin from the solution and the precipitate is readily resuspended. The action of benzidine heparin administered subcutaneously in large doses to anaesthetized dogs is much more uniform and prolonged than that of the same amount of unmodified heparin. It is possible to heparinize the anaesthetized dog for some forty hours by one subcutaneous injection of four cubic centimetres of this preparation of benzidine heparin.

In normal unanaesthetized animals the rate of absorption of unmodified heparin or of benzidine heparin is much more rapid than in the anaesthetized animals. This finding has led us to investigate the effect of muscular exercise upon the absorption of several other substances which are of interest to physiologists.

The fact that general heparinization for prolonged periods can be produced by the subcutaneous administration of solutions of the anticoagulant has made feasible the study of the effect of heparin upon thrombosis of blood vessels in small animals such as, for example, that produced in the tail of the rat by the administration of ergotamine.

*Brain potentials and depth of sleep.* HELEN BLAKE (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Chicago, Ill.

Human brain potentials were led from metal disc electrodes on the intact scalp over occiput and forehead through a resistance capacity-coupled amplifier into loud speaker, cathode ray oscillograph, crystograph or some pair of them. Records were taken through twenty-two nights of sleep on seven subjects, during daytime napping on eight and during sleep after insomnia up to 60 hours on five. Sleep depth was measured by the duration of a sound of constant intensity necessary to awaken the subject, as well as by less quantitative criteria.

The results indicate that potential patterns correlate with sleep depth not only over long-time (hour) changes but, in most cases, over short-time (minute) swings as well. Deep sleep is associated with large, regular potential waves at  $\frac{1}{2}$ -3/sec., lighter sleep with feeble irregular potentials or with the 10/sec. rhythm. Details of transitional potentials from light to deeper sleep, and especially during spontaneous or stimulated awakening are described.

The depth of sleep curve and the potential patterns of daytime sleep (which is usually light) and sleep after insomnia (which is deep) confirm the results on regular night sleep.

These findings are interpreted in terms of excitation levels of cerebral neurones and of the play of afferent impulses upon them. With low

excitation a slow rhythm is manifest, with stronger excitation a faster one, and in transition between these states asynchrony prevents the manifestation of any regular beat.

*Defective development of the cerebellum in the dog.* ROZELLA BLOOD (introduced by O. O. Stoland). Department of Physiology, University of Kansas, Lawrence. (Motion picture.)

Moving pictures with photographs and drawings of the brain are presented of a puppy in which normal coördination of movement did not develop. The brain showed upon autopsy defective development of the cerebellum. This defective development affected the cerebellum alone, the cerebrum being normal. The defect lay in complete absence of the lobus anterior; reduction and distortion of the lobi simplex and ansiformis, flocculus and paraflocculus, and definite deficiency of the lobus medianus posterior. These defects are more pronounced on the left side.

The moving pictures show asthenia on the left side and a marked tendency of the dog to fall to the left, as compared with a litter mate.

*Further experiments on dried natural digestive juices.* W. N. BOLDYREFF, W. B. LEWIS and C. E. STEWART (introduced by W. R. Bloor). Pavlov and Biochemical Laboratories of Battle Creek Sanitarium, Battle Creek, Mich.

Data will be presented on the properties of digestive juices before and after drying. The juices have been dried at 0°C. in an especially constructed vacuum desiccator to prevent autodigestion during the process of desiccation.

*Reflex effects from brief stimulation of the vagus at different stages of the respiratory cycle.* T. E. BOYD and CHARLES J. HILLENBRAND (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

Using dogs under fairly deep anesthesia (barbital-sodium) and with both vagi sectioned, we have applied brief stimuli to the central stump of one vagus. The shocks were just threshold for fibers inhibiting inspiration, and the number of volleys just sufficient to cut short an inspiration at whatever stage desired. The frequency was 120 per second.

Expiration promptly follows the application of such a stimulus, and the ensuing expiratory pause is shortened. The earlier an inspiration is inhibited, the shorter is the interval before the beginning of the next. The reflex effect is limited to one cycle. There is no visible effect unless the stimuli are timed immediately before or during inspiration. A series of volleys sent in during inspiration, but inadequate to inhibit it, has no effect on the frequency. Under these conditions, therefore, shortening of the cycle seems to be an indirect effect of the inhibitory fibers, the center recovering more rapidly following an incomplete discharge, as suggested by Head and by Adrian.

The threshold number of volleys required to inhibit diminishes as inspiration advances, but was never found less than 30.

When similar or even longer periods of stimulation are applied to the superior laryngeal nerve, an inspiration in progress is merely suspended, to be completed after the stimulus is over (Hillenbrand and Boyd, *This Journal* 116: 380, 1936.)

Under lighter anesthesia the effects are irregular, and several cycles may be affected by a brief stimulus to either nerve.

*The rate of emptying of the gall bladder in patients with gastric and duodenal ulcers.* EDWARD A. BOYDEN and THEODORE M. BERMAN (by invitation). Departments of Anatomy and Roentgenology, University of Minnesota, Minneapolis. (Read by title.)

Since the time of Claude Bernard it has been suspected that hydrochloric acid plays a rôle in the evacuation of bile. This has been confirmed for animals but experiments on medical students have raised the question as to whether its effect on the human biliary system is significant. As a further means of testing this hypothesis we have recently undertaken a comparative study of the gall bladder in patients with peptic ulcer, carcinoma of the stomach and pernicious anemia. It will be recalled that the first disease is characterized by an abundance of free acid, whereas the last two are distinguished, respectively, by a decrease and virtual suppression of free acid. The present abstract covers the first of these studies.

Visualization of the gall bladder (oral method) occurred in 30 out of 33 peptic ulcer patients. From this number, 23 good series of cholecystograms were obtained after a standard meal of four egg-yolks and milk. Computation of the changing volumes of the gall bladder has indicated that at least in men under forty, and probably in older men as well, peptic ulcer markedly increases the rate of evacuation of the gall bladder. In considering other factors than hydrochloric acid, which might explain this situation, it was observed that in 9 students who received one egg-yolk placed directly in the duodenum, the mean curve of evacuation equaled that of the ulcer patients. It is suggested, therefore, that the greater motility of the stomach in peptic ulcer cases may be responsible for a greater initial rush of food into the duodenum, which, in turn, causes faster evacuation of the gall bladder. Possibly, also, this vagotomy may relax the sphincter of Oddi. The theory that the large amounts of free acid in the stomach of peptic ulcer patients may also provoke the duodenum to greater production of cholecystokinins is withheld pending a survey of the rate of emptying of the gall bladder in achlorhydric patients.

*The birefringence of muscle.* EMIL BOZLER. Johnson Foundation, University of Pennsylvania and Department of Physiology, Ohio State University, Columbus.

The birefringence of a smooth muscle (retractor muscle of the edible snail) and of the frog sartorius was studied by a new technique. Interference fringes were produced by inserting a compensator wedge between polarizer and analyzer. The muscles, also interposed into this optical path, shifted the fringes and their deviation measured the phase difference of ordinary and extraordinary light rays produced in the tissue. In the methods previously used errors were introduced by the optical inhomogeneity of the tissues and by the necessity of measuring their thickness. However a more accurate measure of birefringence was obtained by integrating the phase differences over a whole cross-section. The quantity so defined was measured by the area between the fringes, produced on the image of the muscle, and the line representing the position of the fringes when the phase difference was zero.

On stretching, the birefringence of the relaxed snail retractor varies

as the square root of the length. The increase of birefringence is not due to a rise of tension, since within the range of lengths used (3.0–6.0 cm.) no measurable tension is produced in the smooth muscle used. During the isometric contraction the birefringence remains unchanged within  $\pm 0.5$  per cent. The changes of birefringence are therefore not associated directly with activity nor with tension, but only with changes of length. Although v. Muralt observed a decrease of birefringence during the isometric contraction in the frog sartorius, the conditions may be fundamentally similar in this type of muscle, because, even in a strictly isometric contraction, the contractile, anisotropic, segments shorten in striated muscle. This view is supported by the observation that only a slight diminution of birefringence is obtained in the frog sartorius at great initial lengths.

*A correlation of the pH of arterial blood and urine, as produced by changes in pulmonary ventilation and by chemical injection.* CHARLES R. BRASSFIELD and VIVIAN G. BEHRMANN (introduced by Robert Gesell). University of Michigan, Ann Arbor.

A correlation of the pH of arterial blood and urine, as followed by a continuous glass electrode arrangement, was made on anesthetized dogs under low oxygen and high CO<sub>2</sub> administration, mechanical asphyxia and chemical injection. Both ureters were cannulated, one being connected to the electrode, and the other to a drop recorder. The urine, flowing through the electrode was collected under oil in 1 cc. tubes for the purpose of checking the continuous method.

Low oxygen often produces a decreased urinary pH, which is usually followed by an increased pH, whereas the blood always shows an alkaline change throughout the administration. Recovery decreases the urinary pH more than the blood pH.

Under CO<sub>2</sub> administration, a sudden decrease in blood pH is compared with a gradual decrease in urinary pH, which culminates at the end of the procedure in a marked acid change of short duration, followed by an immediate alkaline shift. In some instances, a slight increase in pH may occur just after the onset of the procedure, but previous to the gradual acid change.

Urinary secretion is definitely slowed, and, in certain cases, anuria may be produced; with low oxygen (under 10 per cent) and CO<sub>2</sub> (12–16 per cent). However, CO<sub>2</sub> percentages (7–9 per cent) may cause a slight acceleration in flow.

Mechanical asphyxia produces anuria immediately. The urine first secreted, shows a decrease of (0.3–0.6) pH but, within ten minutes, the pH returns to the pre-asphyxia level.

Bicarbonate injections produce an increase in the pH of the blood and urine, although the change is smaller and of shorter duration in the former than in the latter. KH<sub>2</sub>PO<sub>4</sub> in amounts insufficient to cause little change in blood pH produce a sharp decrease in urinary pH with a gradual return to the pre-administration level.

*Extremely prolonged survival of marmots after nephrectomy or adrenalectomy.* S. W. BRITTON and H. SILVETTE. Physiological Laboratory, University of Virginia. (Read by title.)

The marmot (*Arctomys monax*) has been found to be able to withstand

bilateral nephrectomy or adrenalectomy for extremely long periods. The phenomenon is observed during the winter months only but is not explicable on the basis of hibernation, since this animal under laboratory conditions is normally active throughout the year.

After winter-removal of both kidneys the marmot appears in normal health, eats and drinks well and may gain weight, maintains its body temperature and fights vigorously with its cage-mates, for periods up to 30 or 35 days. Death eventually occurs with a very high blood urea, sometimes over 1 per cent, and other signs of uremic intoxication. In summer-operated animals survivals have in contrast averaged only a few days, in agreement with observations on other forms.

Survival after adrenalectomy is also related to the season of operation. Marmots which are adrenalectomized in winter usually survive until spring, when death occurs (as previously reported) with the ordinary symptoms of insufficiency. Thus, early winter-operated animals may survive in good condition (not hibernating) for periods of 100 to 130 days. Summer survival-periods average, however, slightly less than a week.

A number of chemical changes in blood and tissues have been observed under the above conditions, and comparison made with the reactions found in other species.

*Observations on adrenalectomized dogs.* S. W. BLITTON and H. SILVETTE.  
Physiological Laboratory, University of Virginia.

Blood and tissue changes have been studied in an extended series of adrenalectomized dogs under different conditions. While kept on small amounts of adrenal extracts prepared in this laboratory, normal blood conditions were well maintained. No administration of salt and no dietary precautions were found necessary.

Withdrawal of extract resulted in reduction of blood glucose, blood sodium and chloride, and increase of blood urea and potassium. None of these changes appeared to be sufficiently severe to cause death. The blood glucose changes nevertheless appeared very significant: in 25 animals showing various symptoms of adrenal insufficiency the average of all readings was 57 mgm. per cent. This constituted a blood glucose reduction of over 60 per cent from the normal. Tissue analyses in 8 of these cases showed the liver glycogen to be reduced 85 per cent and the muscle glycogen 42 per cent.

Administration of extract to adrenalectomized dogs showing symptoms of insufficiency resulted in an early rise in blood glucose simultaneous with a fall in blood urea. Not until much later were changes in other blood constituents observed.

Severe prostration of the adrenalectomized dog has been frequently overcome by the administration of glucose or glucose-saline solution. A dying animal may thus be restored to an apparently normal condition within 10 to 15 minutes after injection. The restoration is, however, only temporary in nature. Saline solutions show no such restorative effect on animals with severe insufficiency.

*Cerebellar conditioned reflexes.* WILFRED J. BROGDEN (by invitation) and W. HORSLEY GANTT. Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md. (Demonstration.)

In acute experiments a point was found in the lateral cerebellar lobe

beneath the cortex giving on faradic stimulation a predominant ipsilateral foreleg movement. To study systematically this and other cerebellar movements in the chronic preparation this point was located by the external skull markings, and electrodes leading from a subcutaneous induction coil were inserted so that this movement could be regularly elicited at will in the normal animal (dog) by stimulation from an external field coil.

Weak faradization evoked an isolated ipsilateral foreleg extension. Increasing the current amplified the foreleg movement and brought in the ipsilateral hindleg; still stronger stimulations involved all four legs. The animals made no general orientation nor evinced signs of "awareness" unless the current was strong enough to cause struggling. Pain was absent except with currents sufficiently intense to produce violent defense reactions had the current been applied to the skin.

Using this foreleg extension to cerebellar stimulation as the unconditioned reflex we elaborated after five to fifteen trials a conditioned reflex to an auditory stimulus preceding the cerebellar shock by 2". Elaboration occurred as quickly as do conditioned reflexes from a painful skin stimulus—notwithstanding complete absence of signs of pain. The patent period was short—less than 0.5 second.

The position of the electrodes shown by x-ray and at autopsy (within the lateral cerebellar lobe and well beneath the cerebellar cortex) as well as the character of the responses is evidence that the movements were not due to current spread to the brain stem but was primarily caused by cerebellar stimulation. The rapid formation and the stability of the conditioned reflex indicates that it was not elaborated to proprioceptive stimuli resulting from the stimulation. In the light of this apparent integrative function of the cerebellum further researches will be directed to the relation of this organ to the higher nervous structures—viz., the thalamus and the cerebral cortex.

*The influence of dextrose and of increased metabolism on the utilization of  $\beta$ -hydroxybutyric acid.* R. H. BROH-KAHN (by invitation) and I. ARTHUR MIRSKY. Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati, O.

The ketone content of the blood is dependent upon the difference between the rate of ketone formation and the rate of ketone utilization. A reduction in ketonemia consequent to carbohydrate administration must be due either to a decrease in the former ("antiketogenesis") or to an increase in the latter ("ketolysis"). From this point of view it is still unsettled as to whether carbohydrate is antiketogenic in that it prevents the formation of ketones, or ketolytic in that it accelerates the utilization of these substances.

In order to determine which of these is the responsible factor, we studied the rate at which injected  $\beta$ -hydroxybutyrate is removed from the blood of normal and eviscerated rabbits.

Our study demonstrates that:

1. The utilization of  $\beta$ -hydroxybutyric acid is no greater in the normal nephrectomized animal than in the eviscerated preparation. Therefore, the liver does not utilize ketone bodies;
2. The administration of glucose does not increase the rate at which  $\beta$ -hydroxybutyric acid is removed from the blood of either normal or eviscerated rabbits. Therefore, glucose is not ketolytic in action;

3. There is no constant molecular ratio between the removal of glucose and that of ketone bodies by the extrahepatic tissues. Therefore, any "ketolytic ratio" is probably fortuitous.

In view of the fact that liver is the main site of ketone formation and that it does not utilize these substances, it is probable that they represent the end-products of fat metabolism in the liver. Furthermore, the effect of glucose in decreasing diabetic or non-diabetic ketosis must be due to a suppression of ketone formation, i.e., an inhibition of fat catabolism in the liver ("fat sparing" or antiketogenesis). In other words, instead of facilitating fat oxidation, carbohydrate actually inhibits it.

It was also demonstrated that dinitrophenol or thyroid administration accelerates the rate at which the ketones and glucose are removed from the blood by the extrahepatic tissues. Since an increased metabolism also may stimulate ketone body formation in the intact animal, it is obvious that the resultant ketonemia will be dependent upon the relative changes in the rates of ketone formation and utilization.

*The effects of activity and altered circulation on ganglionic transmission.*

D. W. BRONK and M. G. LARRABEE (by invitation). Johnson Foundation, University of Pennsylvania, Philadelphia.

The mechanism of transmission through the stellate ganglion in its relation to neuro-humoral theories has been studied by stimulating one or more of the preganglionic roots while recording the discharge in the postganglionic nerve. The circulation has been controlled by perfusing with blood or oxygenated Ringer's.

In a circulated ganglion submaximal preganglionic stimulation at a frequency of 5 to 10 a second produces a discharge of postganglionic volleys with a corresponding frequency and of a constant size. Ten to fifteen minutes after the circulation has been stopped similar stimulation produces a series of postganglionic responses which progressively increase in size until they become 4 or 5 times as large as the initial volley of the series. Furthermore, if the stimulation be continued for some seconds and then stopped, a preganglionic volley 30 seconds later will still evoke a postganglionic volley larger than the initial test response. Here is evidence for long-lasting facilitation in non-circulated ganglia.

When the preganglionic nerve is stimulated at a frequency of 30 or more a second the postganglionic spike potential rapidly decreases in size. Following a period of such stimulation a condition develops which causes more ganglion cells to respond to a given stimulus. This reaches a maximum after some seconds when the postganglionic response may be 4 to 6 times as great as a test response preceding the period of activity. This effect is more marked in a ganglion without circulation.

The postganglionic response to a single preganglionic test volley has been recorded before and after a train of antidromic impulses. For several seconds after the last antidromic impulse a larger number of ganglion cells respond to the preganglionic volley. This suggests that some of the long-lasting effects which increase the ganglionic response are associated with altered properties of the ganglion cells themselves.

About 30 minutes after the circulation of a ganglion is stopped the ganglion cells begin to lose their capacity to respond, all failing after some 60 minutes. If the perfusion is again started successive cells regain their irritability, and the response is again maximal after 10 minutes.

*Localized faradic stimulation of the medulla oblongata and its effects upon breathing.* JOHN M. BROOKHART (by invitation), ELLIS H. STEFFENSEN (by invitation) and ROBERT GESELL. University of Michigan, Ann Arbor. (Read by title.)

Needle electrodes bare at the tips and separated 1 mm. were directed to miscellaneous points in the brain stem and brief localized faradic stimulations applied. Accompanying changes in costal and abdominal breathing and of tidal air were recorded along with local action potentials before and after stimulation. After each observation a small electrolytic lesion was placed and the electrodes withdrawn. Data from 100 observations appear in the table. In each column under results, the first figure represents the number of observations, the second the incidence of respiratory potentials and the third the incidence of differential effects on costal and abdominal breathing. Further data are considered essential for interpretations.

LOCATION OF STIMULATION	SLOWING FOLLOWED BY		ACCELERATION FOLLOWED BY		NO RATE CHANGE	
	Acceleration	Normal rate	Acceleration	Normal rate	Decreased amplitude	Increased amplitude
Nucleus of Desc. Root of V and Desc. Root of V...		3 0 0	6 0 5	3 0 1	1 0 0	
Nuclei Gracilis, Cuneatus, Corpus Restiformis and Tracts.....	3 2 0	3 0 0	3 0 1	2 0 0		
Nuclei of the Vestibular Group.....			2 1 0	3 0 3		
Region of Fasc. Solitarius and Dors. Efferent Nucl. of X.....	2 1 0	1 1 0				
Fasciculus Solitarius and its Nucleus.....	3 1 2	3 2 0				
Reticular Grey Formation.....	11 4 4	8 2 0	3 1 2	1 0 0	2 1 1	
Lateral Reticular Nucleus.....	2 1 0	1 1 1	1 1 0			
Medial Reticular Nucleus.....	2 0 2	2 0 0	2 0 1	3 1 2		
Nucleus Ambiguus.....	2 1 2	1 0 1	2 1 2			
Dorsal Efferent Nucleus of X.....	1 1 0	2 0 1				
Nucleus Hypoglossus.....	2 1 2	1 1 1				
Medial Reticulo-Spinal Tract.....	3 0 0				1 0 0	
Ventro-Medial Cell Column.....			2 0 2			
Pyramid and Crossing Pyramidal Fibers.....		1 0 0		4 0 0		
Longitudinal Fiber Tracts in Ventral Funiculus.....	1 0 0			1 0 0		

*Studies on the neural basis of ovulation in the rabbit.*<sup>1</sup> CHANDLER McC.

BROOKS. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

Entire removal of the sympathetic chains of female rabbits does not prevent ovulation on coitus. If a reflex stimulation of the anterior lobe is essential to ovulation some nervous pathway other than the sympathetic must carry impulses to the pituitary.

Fourteen rabbits with severed pituitary stalks failed to ovulate though they mated frequently. Ten of these had intact sympathetic systems. There were no indications of pituitary abnormality, other than this failure to ovulate on coitus. Body weights and growth were normal; the genital tracts, ovaries, thyroids and adrenals were of normal weight and showed no histological abnormalities; changes in vaginal smears were associated with the periods of mating; there was no increased sensitivity to insulin and sugar tolerance curves were normal; follicles ripened in the ovaries and ovulation occurred on injection of pregnancy urine.

The venous plexus of the stalk was necessarily severed during the

<sup>1</sup> Aided by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.

operation. Carmin gelatin and india ink injections of the pituitaries showed slight circulatory abnormalities. Histological studies revealed that degenerative changes in the posterior lobes had occurred. The eosinophils and basophils of the anterior lobe were fewer in number and in many of them degranulation of cytoplasm had occurred. Normal cells were present in considerable numbers. Five animals with partially transected stalks mated and ovulated although their pituitaries had undergone cellular and circulatory changes comparable to those observed in the fourteen rabbits with completely transected stalks.

Various silver impregnation techniques revealed fibers resembling nerve fibers in the anterior lobe of normal pituitaries. These fibers were present in four rabbits which had ovulated after partial stalk transection, but could never be found after complete stalk transection (8 animals).

It is felt that these experiments support the hypothesis that normally a nervous stimulation of the pituitary occurs on mating and that the nerves involved pass down the stalk to the anterior lobe.

*Parenteral protein in pneumonias.* CLYDE BROOKS. Louisiana State University Medical Center, New Orleans.

Twelve hundred cases of pneumonia, including all forms, and all ages, were treated with our best routine, including hygienic regimen, nursing, medication, and surgery when indicated.

One half of the cases were used as controls, while the other half were treated by parenteral administration of deuterio-proteose.

The results show for the controls a death rate of 39 per cent for lobar pneumonia, and 32 per cent for broncho-pneumonia. The death rate for the cases treated with deuterio-proteose is 10 per cent for lobar pneumonia, and 9 per cent for broncho-pneumonia.

The results are encouraging, if not conclusive. They indicate that the parenteral use of deuterio-proteose reduces the death rates in both lobar and broncho-pneumonia.

*Agents which antagonize the curare-like action of magnesium.* JOHN J. BROSNAN (by invitation) and T. E. BOYD. Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

Using cats and dogs, under nembutal anesthesia, we have recorded twitch responses of the tibialis anticus and tongue muscles to indirect stimuli at uniform slow frequencies of 10 to 15 per minute. After "curarization" with  $MgSO_4$ , any one of the following agents will induce more or less complete recovery:

$CaCl_2$  (0.5 to 2.0 cc. of isotonic solution)  
KCl (0.5 to 2.0 cc. of 4 per cent solution)  
Acetyl choline (0.1 to 1.0 mgm.)  
Adrenalin (0.01 to 0.1 mgm.)  
Eserin (0.5 mgm. per kilo)

The first three act most quickly and effectively on injection directly into the artery leading to the muscle. The last two act more slowly, and intravenous injection is nearly or quite as effective as intra-arterial. This suggests that the action of adrenalin is indirect, and possibly due to mobilization of K. So far we have used adrenalin only with cats.

No evidence of antagonism between Ca and K has been noted. Moderate doses of the two, combined, induce a greater degree of recovery than either alone.

*Survival of excitability in excised muscle.* E. H. BRUNQUIST (introduced by R. W. Whitehead). Department of Physiology and Pharmacology, School of Medicine, University of Colorado, Denver.

Conditions which tend to prolong the survival of excised muscle in non-nutrient physiological solutions have been sought in an approach to the general problem of the nature and vigor of the vital processes which maintain excitability following excision.

By the exercise of a moderate amount of care, sartorii from the general run of frogs received from biological supply companies have been kept alive (under moderate tension) in a modified Ringer solution for surprisingly long periods. Now and then a shipment of frogs is in too poor a condition to warrant use.

Following are data for three temperature ranges showing the life span of muscles in modified Ringer solution free of any bacteriostatic agent:

TEMPERATURE	NUMBER OF MUSCLES IN GROUP	LONGEST SURVIVAL	SHORTEST SURVIVAL	AVERAGE LIFE-SPAN OF MUSCLES OF MEDIAN THIRD OF GROUP
°C.		days	days	days
5-6	70	142	8	26.4
13-14	100	114	3	12.0
25	54	12	1	4.6

Thus far, boric acid has been the most useful bacteriostatic agent. With its use there is less variation in the period of survival of the two muscles of a pair.

Opinion and data are solicited on the question of ways in which vital activities in excised muscle differ from those in the normal undisturbed tissue, and concerning bacteriostatic agents for use with muscle.

*The effect of anterior cordotomy on spasticity of the skeletal muscles in man.*

PAUL C. BUCY. University of Chicago, Chicago, Ill.

Animal experimentation has indicated that the diminution of the extensor spasticity and the postural reflexes of decerebrate rigidity caused by severance of the spinal cord is largely dependent upon interruption of the vestibulo-spinal tracts. Because of these observations section of the vestibulo-spinal tracts, in the antero-medial columns of the spinal cord, was undertaken in three children suffering from congenital spastic paralysis. It was hoped that this procedure would diminish the spasticity and thus permit greater freedom of voluntary movement. Graphic records of the resistance to passive movements present in the involved extremities were made both before and after operation. Immediately following section of one antero-medial column there was some reduction in spasticity in both lower extremities. This did not persist, however, and the pre-operative condition soon obtained. Section of both antero-medial columns also failed to produce any persistent change.

*Further studies on the cause of the calcification of the crystalline lens.* W. E.

BURGE, G. C. WICKWIRE (by invitation), H. W. NEILD (by invitation) and F. M. HILPERT (by invitation). Department of Physiology, University of Illinois, Urbana.

A quartz tube, 8 cm. long and 6 mm. in diameter, was filled with freshly macerated pigs' lenses. A platinum wire was inserted into the lens material in each end of the tube and the projecting ends of the wires were connected with a delicate galvanometer. The quartz tube was then placed on ice and the whole preparation covered with cardboard except for one platinum electrode and surrounding lens material. This and similar preparations were irradiated with ultra-violet from a large quartz-mercury burner, as well as with sunlight and infra-red. The temperature of the lens material never rose above body temperature, 37.5°C.

It was found that the radiation from all the sources caused the lens material to become electronegative. Ultra-violet was much more effective than sunlight, while infra-red had only a slight effect. Interposing of the cornea which cuts out wave-lengths shorter than 302  $\mu$  decreased but did not abolish the effect of sunlight and of ultraviolet from the quartz mercury arc. The application of a weak solution of calcium chloride abolished the electronegativity of the irradiated lens material, while sodium phosphate restored it. The presence of phosphate in the fresh lens material was demonstrated with the use of ammonium molybdate paper.

It is known that ultra-violet possesses ionizing power and it is assumed that the short wavelengths cause ionization of the lens material, particularly the phosphate, and that this combines with the calcium normally present in the lens to precipitate insoluble calcium phosphate, thus producing calcification of the lens. Such a mechanism of calcification offers an explanation for the fact that the calcium in senile cataractous lenses occurs principally as calcium phosphate. The prevalence of cataract in the tropics, according to this hypothesis, is due to the relative richness of tropical sunlight in ultra-violet. The slow calcification of the human crystalline lens with advance in age resulting in a gradual receding of the near point of distinct vision, until at 70 years all power of accommodation is lost, is attributed to the action on the lens of the small amount of ultra-violet in daylight and artificial illuminants.

*Sorbitol as a diuretic.* G. E. BURGET, W. R. TODD (by invitation) and E. S. WEST (by invitation). Departments of Physiology and Biochemistry, University of Oregon Medical School, Portland.

The diuretic properties of a 50 per cent sorbitol solution and the rate of its elimination have been studied in anesthetized and normal dogs. Repeated injections of 50 cc. over a period of several days gives no evidence of any toxic effects. In dogs under nembutal anesthesia the injection of 50 cc. of a 50 per cent solution produces a much greater diuresis than a like amount of sucrose. When slowly injected there is no noticeable effect upon blood pressure or respiration. The concentration of sorbitol in the blood stream and in the urine obtained by catheterization has been followed after injecting 50 cc. of a 50 per cent solution into two normal dogs. These observations indicate that the concentration in the blood stream falls very rapidly during the first hour after which its removal takes place quite slowly and is still present after twenty-four hours. The amount found in

the urine corresponds with this in that the first two hour specimens have a high concentration of sorbitol.

It has been used clinically in a few cases with indications of being superior to other physical diuretic agents.

*The motor mechanism of shivering and of thermal muscular tone.* A. C. BURTON (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

Records of the activity of single and of multiple units of the muscles of Nembutalized cats have been made, using needle electrodes, when the body temperature was lowered by a cooling tube in the stomach.

When the rectal temperature falls below a critical level, from 37°C. to 35°C. depending on the depth of anesthesia, intermittent and uncoordinated twitches of single units begin to appear. This is coincident with the first signs of a rise in oxygen consumption. This response to cooling is first seen in the muscles of head and thorax, later in the superficial pelvic muscles and finally in the deep muscles of the extremities. The response of a given muscle increases *a*, by increase in the regularity and in the frequency of single units from about 5/sec. up to 12/sec.; *b*, by the number of units active. In the later stages, coordinated grouping of the discharges of units appears and results in the gross tremor of shivering at about 11/sec., a single unit discharging only once in each group. The rôle of the reflex arc in producing this grouping is discussed. The grading of intensity is compared with that of voluntary contraction, where the higher maximum frequency of units leads to greater development of tension for the same heat production.

The response is greatly influenced by posture, due to facilitation when a muscle is stretched. The pattern of ipsilateral and contralateral reflexes can be shown upon the background of thermal muscular tone.

The electrical activity of the muscles is a sensitive index for study of the effect of various agents upon the cold-response. These have included ether, CO<sub>2</sub>, anoxia, amyl nitrite, adrenalin and antipyrine.

*Changes in plasma carbon dioxide combining power and serum calcium in normal and gastrectomized dogs.* R. A. BUSSABARGER, WM. B. BRADLEY and L. G. LEDERER (introduced by A. C. Ivy). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

In a previous report (Proc. Am. Physiol. Soc., 1936) gastrectomy in young puppies was noted to produce deformity and decreased calcification of bone. Although repeated determinations have shown the serum Ca, P, and phosphatase to be normal, the bone changes continue to appear. Since the solubility of Ca salts is increased and the likelihood of calcium deposit is diminished by decreasing alkalinity, determinations of the carbon dioxide combining power of the plasma and the calcium of the serum were made. Samples were drawn from animals fasted 24 to 36 hours and then one, two, and three hours after feeding meals consisting of boiled hamburger and milk; meat and cereal; meat, cereal and milk; and boiled beef heart. The results of these determinations are summarized in the following table.

MEAL	CARBON DIOXIDE COMBINING POWER, CC./100 CC. OF PLASMA						
	Animals	Number of animals	Number of determinations	Fasting sample	1 hour	2 hours	3 hours
Average of all meals	Gastrectomized dogs	6	15	46.1	40.2	42.0	42.9
	Normal dogs	3	7	46.9	51.3	53.1	51.9
SERUM Ca, MG./100 cc.							
Meat, cereal and milk	Gastrectomized dogs	3	3	10.8	12.8	11.6	11.3
	Normal dogs	2	2	11.5	10.0	10.4	10.2
Meat, cereal and water	Gastrectomized dogs	3	3	10.3	9.6	9.5	9.4
	Normal dogs	2	2	11.1	9.9	9.8	9.9
Boiled beef heart and water	Gastrectomized dogs	6	10	9.1	8.5		8.3*
	Normal dogs	3	4	9.4	9.0		9.1

\* Only 6 determinations.

*The transmission of impulses through a sympathetic ganglion.* W. B. CANNON and A. ROSENBLUTH. Harvard Medical School, Boston, Mass.

The superior cervical sympathetic ganglion (s.c.g.), when perfused with salt solution and stimulated from preganglionic (pre-g.) fibers, gives off acetylcholine (a-ch.) into the perfusate. A-ch. in small concentration stimulates; in larger, paralyzes. Curare raises the thresholds for both stimulation and paralysis. Eserine or prostigmin protects a-ch. from quick destruction by an esterase. Rapidly repeated stimulation of the ganglion causes a large initial outburst of a-ch., followed by a much smaller production. Evidence for the foregoing statements has been obtained mainly by Dale, Feldberg and their collaborators.

Prostigmin, given shortly before tetanic stimulation of pre-g. fibers, cuts short the contraction of the n.m. and induces relaxation; as minutes pass the depressant action gradually disappears. The relaxation is attributed to excess of a-ch., preserved from prompt destruction by prostigmin.

Prostigmin, injected after tetanic stimulation of pre-g. fibers has failed to maintain the n.m. in full contraction, causes an increase of contraction. If the lower contraction resulted from "fatigue" (i.e., insufficient a-ch. to activate some ganglion cells), the increase is explicable as a preservation of a-ch. until more cells are activated.

Injection of a-ch. during the fatigue phase has an effect similar to that produced by prostigmin, and can be explained on the same basis; injected at the start of tetanic stimulation, a-ch., like prostigmin, quickly interrupts contraction and induces relaxation. Again the action, like that of prostigmin, is attributable to a depressant excess of a-ch., at this phase.

Either a-ch. or prostigmin has a decurarizing action on the ganglion. The explanation is found in the action of curare as raising the threshold of stimulation. The fatigue effect is magnified (i.e., the level of contraction falls); a-ch., by supplying an extra quantity, and prostigmin, by protecting from destruction the a-ch. which is naturally produced, raise the stimulus above the raised threshold.

A brief tetanus, introduced in a series of maximal single shocks, causes the succeeding contractions to be much higher than those which preceded. Probably the tetanus releases in the ganglia an adjuvant (perhaps potassium) of a-ch., which results in repetitive discharges from single pre-g. volleys.

*Thyroid deficiency in the mother and its effect on the thymus of the new-born white rat.* D. BAILEY CALVIN and KENNETH KAUFMAN (by invitation).

Department of Biochemistry, University of Missouri School of Medicine, Columbia.

In a previous report (Proc. Soc. Exper. Biol. and Med. **34**: 724, 1936) data were presented in support of an inter-relationship between thyroid condition of the mother and thymic size in the new-born white rat. Due to a number of difficulties attendant upon the post-operative conditions following thyroidectomy in the breeding females, the evidence for thymic change in the fetuses from hypothyroid mothers was not covered in any detail. The present report deals with this problem and with post-operative care of thyroidectomized animals.

In the earlier experiments, partial or complete thyroidectomies were performed on mature female rats of proven breeding and rearing quality. After operation the animals were maintained *until bred* (7 to 10 days) on a high calcium intake. It was found that these animals had hypocalcemic complications beginning about the thirteenth day of pregnancy. A number died between the fifteenth and eighteenth days of gestation. In a later series of experiments, all animals were maintained throughout gestation on either a high calcium dietary or parathyroid extract (Lilly) or both.

In general, breeding and maintenance results were more satisfactory where partial rather than total thyroidectomy was performed, care being taken to avoid injury to the recurrent laryngeal nerve.

The following results were obtained, using totally or partially thyroidectomized females, and proper post-operative care: (1) The gestation period was longer than normal, usually from 22-25 days. (2) Parturition was quite difficult, in one case lasting for 28 hours. (3) A high percentage of the operated females died during or shortly after parturition, unless the above mentioned precautions were taken to compensate for hypo-parathyroid complications. (4) The average thymic weight per gram of body weight of the new-born fetus was about 20 per cent greater than for fetuses from normal breeding females in the colony. (5) Partially thyroidectomized females gave more satisfactory results. Fetuses from these animals had thymus glands as much as 50 per cent above normal weight.

Preliminary clinical studies are now in progress in an endeavor to discover whether or not these data have any bearing on the debatable problem of status-thymico-lymphaticus.

*Carbohydrate metabolism in the hypophysectomized depancreatized dog.*

WILLIAM H. CHAMBERS, J. E. SWEET and J. P. CHANDLER (by invitation). Departments of Physiology and of Surgical Research, Cornell University Medical College, New York City.

Glucose oxidation in the "Houssay" dog has been studied in the respiration calorimeter when the animals were maintained on an adequate diet of meat and carbohydrate or meat alone. The post-absorptive respiratory

quotients in 19 experiments averaged 0.76, an indication of some carbohydrate oxidation. Individual animals varied from 0.73 to 0.82.

Despite the elevated basal quotient, the ingestion of 50 grams of glucose caused no significant rise in R.Q. in 9 of the dogs. In 3 other animals an increase of about 0.05 was observed. From 20 to 80 per cent of the ingested sugar was recovered in the urine.

Carbohydrate balance studies were made on 2 animals by giving one-half of a constant diet every 12 hours and determining the respiratory metabolism for 8 hours during the day. The body weight was maintained at a constant level. On a food intake of meat and pancreatin for 5 days one of the animals responded like the depancreatized dog with average respiratory quotients of 0.72 and a D:N ratio of 3.0. The other gave evidence that some of the glucose from the ingested protein was oxidized, since the quotients averaged 0.75 and the D:N ratio was 2.5. In both animals, when 30 grams of glucose per day were added to the meat diet for 5 days, about 16 grams per day were not recovered in the urine. The rise in the quotients to 0.78 is consistent with the oxidation of this amount of glucose. Increasing the glucose in the diet to 100 grams per day gave no indication of a greater ability to metabolize the glucose or retain it as glycogen, since the R.Q.s remained at about 0.78, and 86 per cent of the 100 grams was excreted.

Serial sections showed only minute amounts of pars tuberalis in some dogs; in others small pieces of anterior lobe tissue were found. No correlation between the amount of suspected tissue and the metabolism of glucose could be established.

The results indicate that the carbohydrate metabolism of the "Houssay" dog is maintained at a relatively low level.

*The action and toxicity of gelsemicine.* K. K. CHEN and T. Q. CHOU (by invitation). Lilly Research Laboratories, Indianapolis, Indiana, and Institute of Materia Medica, Shanghai, China. (Read by title.)

Gelsemicine is a new alkaloid isolated by one of us (T. Q. C.) from the roots of *Gelsemium sempervirens*. It has an unusually high toxicity. The minimal lethal doses of its hydrochloride in milligram per kilogram determined by intravenous injection are 0.09 in mice, 0.125 in rats, 0.35 in guinea pigs, 0.06 in rabbits, and 0.2 in cats. Monkeys show signs of poisoning with doses of 0.2 to 0.07 mgm. per kgm. Toxic symptoms usually appear after a long latent period. Death is apparently due to the paralysis of the respiratory center, for pithed cats can tolerate several otherwise fatal doses.

*On the birefringence of nerve cells.* PRISCILLA CHINN (introduced by F. O. Schmitt). Department of Zoology, Washington University, St. Louis, Mo.

Unlike the axis cylinder, the cytoplasm of nerve cells is packed with granules and inclusions of various sizes. Nevertheless, examined fresh in Ringer solution, frog spinal ganglion cells show negative polarization crosses extending throughout the cytoplasm. In motor horn cells the cytoplasm is positively birefringent with respect to the distinguishing directions of the multipolar cells. This picture is not materially affected by treatment with alcohol, and immersion in media of high refractive index does not reverse sign of birefringence. These effects would be explained

on the assumption of the preexistence of protein micelles comparable to those present in the axis cylinder but oriented, generally speaking, in the manner indicated by the course of the neurofibrils revealed in such cells by classical histological methods.

Unipolar ganglion cells of the ventral cord of the leech and crayfish are provided with an enclosing sheath which is continuous with the lipoid containing axon sheath, and, like the latter, shows the metatropic reaction, indicating a fundamental similarity of ultrastructure with respect to the lipoid and protein micelles.

Frog spinal ganglion cells are also surrounded by a layer containing a small amount of oriented lipoid. This metatropic sheath may be seen as a thin layer surrounding the axon from the region of the cell body to the beginning of the myelin sheath. The inner layer of the capsule also gives the metatropic reaction and is distinct from the outer connective tissue layers as shown by its failure to swell in dilute alkali. The presence of lipoid is also indicated by profuse production of myelin forms from cells teased in dilute glycerine or alkaline solutions.

*Concerning the use of carotene as a liver function test.* BYRON B. CLARK, J. B. ROBINSON and L. J. SCHIFF (introduced by H. E. Himwich). Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. (Read by title.)

Abnormally high blood carotene tolerance curves have been reported in cases of diabetes mellitus following doses of 60 mgm. carotene in oil. The explanation suggested was that the ability of the diabetic liver to convert carotene to vitamin A is diminished. It seemed desirable to determine blood carotene following carotene ingestion in non-diabetic patients with liver disease, and in dogs with experimentally produced fatty livers.

Two patients with cirrhosis of the liver were given 60 mgm. of carotene in oil. Blood samples were drawn at 0, 4, 10 and 24 hours for carotene determination. These curves were not significantly different from reported curves for normals. Positive evidence of liver insufficiency was given by the bilirubin injection, and bromsulphalein liver function tests.

In four experiments, dogs were fasted and given phlorhizin. Under such conditions they develop fatty livers averaging about 10 per cent fat. Such animals show positive bromsulphalein and bilirubin retention. Tolerance curves performed by giving these animals 30 mgm. of carotene in oil by mouth showed no greater increases in blood carotene values than the animals own control curves.

One dog was given 16 mgm. per kgm. of phosphorus dissolved in oil by mouth. Thirty mgm. of carotene was given daily for 7 days. The blood carotene values obtained were not different from a control animal fed the same amount of carotene for the same period. The phosphorus-poisoned animal, however, showed positive bromsulphalein retention.

These experiments indicate that carotene in the amounts given and in the conditions tested does not appear to be of value as a liver function test.

*Studies in ketogenesis.* PHILIP P. COHEN (introduced by H. C. Bradley). Department of Physiological Chemistry, University of Wisconsin, Madison.

Twenty-two related fatty, amino, and other substituted fatty acids were studied using rat liver slices in a Warburg-Barcroft apparatus. The rôle of these compounds in ketogenesis and antiketogenesis is reported here.

The data obtained from this study indicates the existence of an oxidative enzyme system, called here beta oxidase system, which requires a specific chemical grouping in the substrate for its oxidative activity. This substrate grouping is reported here as having the following skeleton formula.  $-\text{CH}=\text{CH}-\text{C}=\text{O}$ . The carbonyl group may be present in a carboxyl group, or may exist as such, as in an alpha keto acid, and is considered the polar or orienting part of the group. The adjacent two carbon atoms must have at least one hydrogen atom present on each of the two carbon atoms for oxidation to occur, and may have its full complement of hydrogen atoms, as in propionic acid.

A compound containing the above indicated grouping will be oxidized on the carbon atom beta to the carbonyl group. Thus, alpha keto valeric acid will be oxidized gamma to the carboxyl, but beta to the carbonyl group.

The compounds recently reported by Jowett and Quastel to be specific inhibitors for ketogenesis from known ketogenic compounds, can be shown to contain the specific grouping indicated above. Benzoic acid, one of the strongest inhibitors, contains the group  $-\text{CH}=\text{C}-\text{C}=\text{O}$ , and is therefore

not oxidized by the enzyme system, but is absorbed. This might serve to explain the toxicity of benzoic acid and the necessity of its conjugation for its removal from the body.

The evidence to date on the oxidation of fatty, amino, and other related substituted fatty acids points to a mechanism of successive beta oxidations by the beta oxidase system, each newly formed carbonyl group acting as a polar group for further oxidation beta to the carbonyl group.

The ketogenesis, antiketogenesis, and glucogenesis of the various fatty, amino, and other substituted fatty acids are explainable on the basis of oxidation by the beta oxidase system.

A scheme is proposed indicating a possible mechanism for the oxidative glucogenesis of the odd-numbered fatty acids.

*Experimental pancreatic diabetes in the monkey.* J. B. COLLIP, H. SELYE and A. NEUFELD (by invitation). Department of Biochemistry, McGill University, Montreal, Canada.

The supposition that oestrin has a depressing action on the diabetogenic activity of the pituitary has not received confirmation by our experiments on the pancreatectomized monkey. Both the oestrin-treated (1000 $\gamma$  daily subcutaneously) and the untreated pancreatectomized Macaca Mulatta exhibited hyperglycemia and glycosuria of approximately the same severity. Ketonuria was usually observed during the first few days following the operation, but disappeared later irrespective of whether or not the animal had been treated. Unless complications set in (post-operative pneumonia was quite frequent) this species may survive pancreatectomy for several months without insulin treatment. During this period the animals consumed large quantities of food; but in spite of this they lost considerable weight, the severe atrophy of the muscular system being particularly striking. The changes in the carbohydrate metabolism following pancreatectomy in this species appear to be somewhat similar

to those obtained by simultaneous pancreatectomy and adrenalectomy or pancreatectomy and hypophysectomy in animals such as the dog or the cat.

This became still more evident when we administered insulin to the fasted depancreatized monkey, for we found that it shows much more pronounced hypoglycemia after the administration of this hormone than does the normal. Even without insulin treatment we observed the rather surprising fact that the blood-sugar of the depancreatized monkey, fasted for 34 to 70 hours, decreased to considerably lower levels (22 mgm. per 100 cc.) than that of normal controls fasted for the same period (50-60 mgm. per 100 cc.)

*The relationship of the stage of development of an organ to the effects of x-rays.*

J. D. COMBS (introduced by W. F. Hamilton). Department of Micro-anatomy, University of Georgia School of Medicine, Augusta.

Previous data have shown that the wings of *Drosophila* are going through the active stage of proliferation between the 72nd and the 144th hour of development if the embryos are kept at a constant temperature of 27°C. The experimental procedure of the present investigation was carried out in the Zoological Laboratory of the University of Illinois where the author was a guest during the past summer. Developing *Drosophila* were given 1325 r units of x-rays during different stages of development and observations were made upon the wings of those individuals which emerged. The data were derived from three experimental groups in which the embryos were either x-rayed before, during, or after the proliferating stages of the development of the wings. Results showed that x-raying before and during the proliferating stages produced a significant number of defects in the adult wings. After the period of proliferation the number of defects was probably not different from the control group which was not x-rayed.

*Reflexes from the carotid body (Glomus Caroticum) to the respiratory center of the dog.* J. H. COMROE (by invitation) and CARL F. SCHMIDT. Laboratory of Pharmacology, University of Pennsylvania, Philadelphia.

If branch of occipital artery supplying glomus was patent and innervation of glomus intact, lobeline or cyanide injected into common carotid in minute dosage (0.1 mgm.) caused hyperpnea, but if branch was closed or glomus was traumatized, respiration was unaffected. When external carotid of one side (denervated) was anastomosed with other external carotid (innervation intact) and latter was tied between internal carotid and occipital, lobeline or cyanide injected into denervated carotid reached innervated glomus and stimulated breathing; injected into innervated carotid they had no effect, showing that pressure receptors are insensitive to these agents. When Locke's solution or heparinized blood was pumped through glomus, hyperpnea occurred when O<sub>2</sub> content of perfusing fluid was reduced or CO<sub>2</sub> content increased; changes in pressure had no effect. To test physiological significance blood samples were withdrawn (heparinized) during inhalation of O<sub>2</sub>, CO<sub>2</sub>-O<sub>2</sub>, and N<sub>2</sub> or N<sub>2</sub>O by dogs prepared for perfusion of one glomus; these samples were then pumped through the glomus. In each of 41 experiments in which glomus reacted to lobeline or cyanide anoxic blood stimulated breathing when perfused through glomus and in 29 reflex hyperpnea was greater than hyperpnea produced

by inhalation of gas; latter was entirely absent if both depressor and contralateral sinus nerves were cut. Hypercapnic blood stimulated breathing reflexly in only 21 of these experiments and reflex hyperpnea was much less than that of anoxemia or direct effect of inhalation of  $\text{CO}_2\text{-O}_2$ . Threshold of glomus to increased  $\text{CO}_2$  or decreased  $\text{O}_2$  was about 5 vols. per cent; changes in acidity did not affect glomus, for pH was reduced 0.15 to 0.3 by  $\text{CO}_2$ , increased 0.1 to 0.2 by anoxia, and blood to which acid or alkali was added, changing pH by 0.5 to 1.0, did not affect breathing when perfused through glomus. Section of sinus nerve completely abolished all effects from alterations in perfusion fluid.

Conclusions. 1, reflexes aroused in carotid by chemical agents originate entirely in glomus caroticum; 2, receptors are sensitive both to  $\text{O}_2$  lack and  $\text{CO}_2$  excess; 3, hyperpnea of  $\text{CO}_2$  and acid excess is much more central than reflex, anoxemic hyperpnea mainly or entirely reflex.

*Effects of cortico-adrenal extracts on the carbohydrate metabolism of hypophysectomized rats.* E. L. COREY. Physiological Laboratory, University of Virginia.

Hypophysectomized rats were fasted for 48 hours during which 12 cc. of cortico-adrenal extract were given in four doses. Blood-sugar and liver and muscle glycogen determinations were then made and compared with findings in unoperated controls, hypophysectomized untreated rats, and hypophysectomized rats injected with 0.9 per cent saline solution. All were similarly fasted for 48 hours.

Three types of cortico-adrenal extract prepared in this laboratory were employed. The blood-sugar levels of all extract-injected rats were distinctly higher than those observed in 48-hour-fasted animals as well as in the hypophysectomized untreated controls. They even approximated the average levels observed in unfasted normal rats. The blood-sugar levels were equally well maintained with the highly refined and the relatively crude stock extracts. The results were apparently not due to adrenalin or choline since all assays of the extracts by blood-pressure tests on amyralized cats were negative. No significant effects were observed on the liver and muscle glycogen levels, with the time periods and small dosages of extract employed.

An increase in the blood-sugar level of non-fasted hypophysectomized rats, maintained for about two weeks after operation, was again found in 18 cases, in confirmation of earlier work.

*Effect of insulin and glycine on hepatic carbohydrate metabolism in unanesthetized normal, hypophysectomized, and adrenal denervated dogs.*

LATHAN A. CRANDALL, JR. and IAN S. CHERRY (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Dogs were prepared with cannulae on the portal and hepatic veins according to the technique of London. Observations on the portal, hepatic, and arterial blood sugar, lactic acid, and urea were made after insulin or glycine in normal animals, after removal of one adrenal and splanchnic section on the opposite side, and after hypophysectomy. This technique avoids the abnormalities of carbohydrate metabolism that have been shown to follow anesthesia.

In normal dogs after 15 units of insulin subcutaneously the output of

glucose from the liver increased if the hepatic glucose output had previously been low or moderate. If the hepatic glucose output was high in the control sample, insulin caused a decreased output in 15 minutes with a return toward the normal at 1 hour. Lactic acid changes were variable, urea output was usually increased.

The fasting output of sugar from the liver was less in hypophysectomized than in normal dogs. After insulin the output in both hypophysectomized and adrenal denervated dogs either decreased or did not change. Lactic acid and urea changes were comparable to those in normal dogs. Insulin shock was invariably observed in hypophysectomized and adrenal denervated dogs but not in normals.

After glycine (20 gm. orally), all 3 types of animals behaved similarly. Glucose and urea outputs from the liver were much increased.

Hypophysectomy and adrenal denervation abolish the ability of the animal to respond to insulin hypoglycemia by an increase in hepatic glucose output, but do not appear to affect gluconeogenesis from glycine.

*Mapping the cochlea.* E. A. CULLER, JOHN WILLMANN (by invitation) and F. A. METTLER (by invitation). University of Illinois, Urbana, and University of Georgia Medical School, Augusta.

Two years ago a map of the guinea-pig cochlea was published from this Laboratory, based upon a careful survey of cochlear potentials generated by air-borne frequencies ranging from 60 to 7,000 cycles. This survey differed from all similar efforts in that the cochlea, instead of being drugged, punctured or cauterized, was in every respect normal. The map revealed that each audible frequency has its own *focus of response* within the cochlea, and that the site of a given focal point can be fixed with a standard error of five per cent. To be wholly conclusive, any such chart requires corroboration by hearing-tests after localized lesions. Despite many technical difficulties, such tests have now been made for one critical frequency, 200 cycles, which appears on the chart at mid-ventral aspect of first apical turn. Normal limens are first measured in the pig after destruction of one ear. The cochlear wall is then punctured, opposite spiral ligament at point 200 of the map, with a surgical needle, leaving a tiny perforation which soon heals over. Figures from two representative animals may be cited (hearing-loss at each frequency after lesion):

125 CYCLES	150 CYCLES	200 CYCLES	250 CYCLES	300 CYCLES	500 CYCLES	700 CYCLES	1000 CYCLES
<i>decibels</i>	<i>decibels</i>	<i>decibels</i>	<i>decibels</i>	<i>decibels</i>	<i>decibels</i>	<i>decibels</i>	<i>decibels</i>
17	16	25	22	15	14	9	4
52	53	55	53	52	46	43	39

As typified by these figures, maximal loss always occurs at or very near the charted frequency; the losses however extend over a wide range, all frequencies being impaired. The following general conclusions may safely be inferred.

a. The resonant principle, being now validated by the concurrent testimony of both cochlear response and hearing-tests, seems to be proved beyond any reasonable doubt.

b. These tests provide the first satisfactory evidence of direct agreement between cochlear response and hearing.

c. They indicate that the map previously published is correct.

*Hydrogen peroxide as a depressant of gastric acidity.* C. U. CULMER and A. J. ATKINSON (introduced by F. T. Jung). Departments of Medicine and Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The use of hydrogen peroxide to depress gastric acidity has been reported a number of times in the clinical literature during the past thirty years. Also, its use, in more dilute solutions, as a stimulant of acid secretion has been reported. Owing to these conflicting findings, we deemed it worth while to study its effect under controlled laboratory conditions. Five dogs with Pavlov pouches, three dogs with normal stomachs, and five patients were used.

*Results.* Following lavage with 3 per cent peroxide, five pouch dogs showed a decrease in acid secretion of 56 to 65 per cent to histamine stimulus. After repeated lavage with 3 per cent peroxide, two pouch dogs showed decreases of 77 and 96 per cent to histamine. Three pouch dogs showed decreases of 32 to 83 per cent after  $\frac{1}{2}$  per cent peroxide. Three dogs were used with a meal as stimulus; in two  $\frac{1}{2}$  per cent peroxide markedly decreased acidity, while one showed increased acidity to all strengths of peroxide below 3 per cent. The dogs with normal stomachs showed decreases of 95 to 100 per cent in free acid after 3 per cent peroxide (histamine stimulus); all evidenced marked distress and one suffered serious hemorrhage. Three of the patients showed decreased acidity after 1 to 3 per cent peroxide (only one could tolerate more than 2 per cent), the fourth showed increase to 1 per cent and could tolerate no more, the fifth showed decrease to 2 per cent and his symptoms disappeared. All the patients were distressed by strengths of peroxide above 1.5 per cent; one with gastric ulcer showed marked bleeding.

*Conclusions.* Hydrogen peroxide in solutions varying from  $\frac{1}{2}$  to 3 per cent depresses gastric acidity, its effectiveness increasing the greater its strength. The depressing effect is most marked immediately after lavage, but in some cases persists up to 15 days. The more dilute solutions, however, may stimulate acid secretion. Because of this and since all but the more dilute solutions cause distress and may cause bleeding, we conclude that hydrogen peroxide is not a satisfactory means of depressing gastric acidity in patients.

*The rat in the assay of cortin.* FRED E. D'AMOUR and DOROTHY FUNK (by invitation). Research Laboratories, University of Denver, Denver, Colo.

On the basis of size, ease of handling, numbers available and greater similarity of individuals, one would expect the rat to be a more suitable test animal for the assay of cortin preparations than the dog. However, the suitability of the rat has been questioned by some workers because of reported high percentages of survivals and a difference between different strains in this respect. This paper reports the results of a study of this question.

1. Length of survival and per cent of indefinite survivals. The average length of survival of about 400 uninjected, adrenalectomized albino rats from our colony was 5.9 days; the per cent of indefinite survivals of all rats used (about 700) was 3.8. No significant differences were observed using piebald rats and both figures agree well with Cartland's, who used Wistar rats.

2. Using rats in groups of 5, it is possible to make a statistical assay

the length of survival and weight increase being proportional to the dosage. Results are reproducible, as shown by the agreement of the assay done on the same preparation in different laboratories. The disadvantage in the use of the rat is the large amount of cortin required.

3. Oral administration was consistently more effective than parenteral. This was particularly true of weight increase.

4. Nine commercial preparations were assayed by the rat method. Of the most active preparations, one cc. was equivalent to about 2 rat units. Other preparations, in similar doses, were entirely inactive.

*The assay of gonad stimulating substances.* MARIE C. D'AMOUR, F. E. D'AMOUR and J. D. DUTCHER (by invitation). Research Laboratories, University of Denver, Denver, Colo.

This work embraces a comparison of several methods of assaying gonad stimulating preparations and of the units obtained, a comparison of the activity of preparations from different sources, and a check of certain commercial preparations.

Twenty immature rats were used on each dosage, the total dose given over three days and the animals examined at 100 hours.

Using the increase of weight of organs as a test, a satisfactory unit appears to be the amount necessary to produce seminal vesicles weighing 15 mgm. or ovaries weighing 20 mgm.—an increase of about 100 per cent in each case.

In using the formation of corpora lutea in 30 day females, difficulty in interpretation of results was experienced on intermediate dosages. The unit is taken as the amount giving positive results in 50 per cent.

In the estrus smear test, the state of the introitus must be ignored and all vaginæ smeared. 50 per cent positives constitute the unit.

The weight of organs gives the most objective test, while formation of corpora lutea gives the least. Using the same preparation, the units have the following values in cc. of pregnancy urine: estrous smear, 0.18, corpora lutea, 0.48, seminal vesicles, 0.9, ovaries, 4.8.

With urine, the use of males is more satisfactory, while with pituitary the females are best suited. Ovary curves from urine and pituitary flatten out, but the latter at a much higher level. On males, the placenta curve is essentially the same as with urine. Placenta is a poor source of hormone.

In a study of five commercial preparations, it was impossible to duplicate the manufacturer's assay. Two urine products were close to the stated value, one placental preparation required between two and three times the labelled dosage, while two pituitary products were negative on three and ten times the labelled doses respectively.

*Placental histaminase.* D. N. DANFORTH and FRANK GORHAM (introduced by C. A. Dragstedt). Department of Physiology and Pharmacology, Northwestern University, Chicago, Illinois.

Since histamine is known to be a powerful oxytocic, determinations of histaminase were made upon 84 human placentas by the method of Best and McHenry. Placentas from spontaneous term deliveries, as well as from pathological obstetric cases, were analyzed. Assuming the unit of histaminase to be that amount of prepared powder which will inactivate 1 mgm. of histamine base in 24 hours, it was found that only 4.5 per cent of placentas from women with normal labors contained more than 0.5 unit

per gram of powder, whereas in the group composed of women with sluggish labors, uterine inertias, and caesarean sections before the onset of labor, 50 per cent contained more than 0.5 unit per gram of powder.

*Systolic and diastolic blood pressure continuously recorded: apparatus and applications.* CHESTER W. DARROW (introduced by E. Gellhorn). Institute for Juvenile Research and Department of Physiology, University of Illinois, Chicago.

Devices for the purpose of continuously recording systolic blood pressure have been described by Kolls (1920) and by Kronfeld Müller and Reiner (1933). In the more recently described instrument attempt was likewise made to record diastolic pressure, but this pressure was not critically differentiated. The apparatus here described continuously records either systolic or diastolic blood pressure without puncture of an artery and, with duplicate equipment, it provides simultaneous records of both systolic and diastolic pressure.

In this instrument the changes in the pulse wave below an inflated cuff which are commonly noted by auscultation are utilized mechanically to maintain the critical diastolic or systolic pressure which may be continuously recorded. The effects of the critical change in the pulse wave affect the apparatus through the agency of a second cuff below the one commonly employed in auscultation (or by the lower chamber of a single double cuff). The pulse waves thus transmitted act upon a sensitive "differential tambour" having electrical contacts controlling a vacuum tube relay and an electro-magnetic valve.

For recording diastolic blood pressure the apparatus is so arranged that the pulse waves from the upper and lower cuffs strike opposite sides of the "differential tambour." When air leaks into both cuffs from an air supply, thereby raising the pressure, the pulse wave transmitted from the lower cuff is found at first to be stronger than that from the upper cuff, and when these waves strike opposite sides of the differential tambour an electrical contact remains unbroken. When diastolic pressure is reached the pressure in the upper cuff begins to impede the pulse wave to the lower cuff and in consequence the waves from the upper cuff become stronger than those from the lower cuff. This reverses the differential pressure of the pulse waves on the tambour so that they break the electric contact on the tambour thereby opening the electromagnetic valve and reducing the pressure in the cuffs. This is repeated with each heart beat until the pulse wave from the upper cuff no longer breaks the electrical contact. The pressure in the system is thus held, with only small deviation, at diastolic level.

For recording systolic pressure conditions are reversed so that air is permitted to enter the system at each pulse wave reaching the lower of the two cuffs. This continues until pressure in the upper cuff becomes sufficient completely to cut off the pulse to the lower cuff. A slow "leak" continuously brings the pressure down toward the level where the pulse may again reach the lower cuff, operate the inlet valve, and raise the pressure.

Applications of the apparatus are illustrated by slides showing simultaneously recorded systolic pressure, diastolic pressure and sphygmomanometer pressure under such condition as hyperpnea, apnea, oxygen lack, mental effort (multiplication), embarrassing questions, adrenalin, amyl nitrite, carotid sinus pressure, and muscular tension.

*The measurement of combination tones in the electrical activity of the cochlea.*

H. DAVIS and S. S. STEVENS (by invitation). Departments of Physiology and Psychology, Harvard University, Boston, Mass.

Five ears (2 cats' and 3 guinea pigs') were stimulated by tones of 700 and 1200 cycles. The resulting cochlear potentials were analyzed by means of an electrical wave-analyzer (General Radio), and the magnitudes of the harmonic and combinational components were measured as functions of the intensity of the stimulating tones. With stimulation by either of the primary tones alone at least the first five harmonics could be measured. These harmonics appear after the response to the primary tone departs from linearity.

For combination tones the intensities of the primary tones were adjusted to give equal cochlear responses at a moderate intensity level. The ratio of their intensities was maintained constant while the total intensity was varied. Such stimulation gave, in the ear most thoroughly studied, the following combination tones (letting A represent the lower tone, 700 cycles, and B the upper tone, 1200 cycles):

$B - A = 500$	$2A + B = 2600$	$2A + 2B = 3800$
$B + A = 1900$	$3B - 2A = 2200$	$3A - B = 900$
$2B - A = 1700$	$3A + 2B = 4500$	$3B - A = 2900$
$2A - B = 200$	$3B + 2A = 5000$	$3A - 2B = 300$
$2B + A = 3100$	$2B - 2A = 1000$	

These combination tones are listed (reading by columns) in the order of their appearance with increase of intensity.

As a function of the intensity the more prominent components grow as follows:

1. The sum and difference tones (500 and 1900) grow at about the rate of the fundamental.
2. The second-order sum and difference tones (1700, 200, 3100 and 2600) grow more rapidly—more nearly at the rate of the harmonics—and reach a higher maximum than the sum and difference tones.
3. The remaining combination tones are too small for the determination of reliable intensity functions. At their maximum they do not usually reach a value greater than one per cent of the fundamental.

*Effect of physical training on the osmotic resistance of erythrocytes.* JOHN

E. DAVIS (introduced by R. J. Main). Department of Physiology and Pharmacology, Medical College of Virginia, Richmond.

Erythrocyte osmotic resistance to hypotonic sodium chloride solutions was studied on dogs which were trained by running four miles daily on a treadmill inclined at 25 per cent grade. In four dogs, the osmotic resistance (Hasting's method) was decreased after one week of physical training. The hemoglobin per unit volume of blood was also decreased (10-18 per cent). Previous work has shown that dogs trained by daily swimming exercise showed an increased osmotic resistance of erythrocytes, and it was suggested that the body temperature during exercise may be an important factor in determining these changes. An effort is made to explain the observed changes in red cell fragility.

*The metabolism of lysine: studies on the heat production and blood and urine constituents after administration of d-lysine monohydrochloride to the dog.*

J. R. DOTY (by invitation) and A. G. EATON. Department of Physiology, Louisiana State University Medical Center, New Orleans.

The dogs used in this work were fasted at least 16 hours before the experiment. Two or three determinations of basal metabolic rate and blood amino and urea nitrogen values were made in a control period preceding the administration of the lysine. Blood urea and amino nitrogen levels as well as heat production were determined hourly in a five-hour experimental period. Urine urea, amino, and total nitrogen were determined for the control period and again for the experimental period. Heat production was measured by the Tissot Haldane method, urea by the manometric method of Van Slyke for determining carbon dioxide formed by the action of urease followed by acid, and amino nitrogen by the manometric method of Van Slyke.

Two attempts to administer 8.5 grams of d-lysine monohydrochloride by stomach tube were unsuccessful, the dog vomiting most of the material within an hour. Two experiments were performed by intravenous injection of the same quantities of the amino acid. On one occasion 6.9 grams were excreted in the urine and on the other 6.46 grams were lost in the same manner. Intraperitoneal injections resulted in the excretion of much smaller quantities of the amino acid.

Blood amino nitrogen increased markedly and remained high for some time. Urea nitrogen of the blood was raised little if any. Urine urea nitrogen was increased somewhat. There was little, if any, increase in heat production.

Four control experiments using glycine gave an increased heat production amounting to 0.237 calorie per millimol of glycine metabolized. This value is in good agreement with the results of Wilhelmj, Bollman and Mann and also with those reported previously from this laboratory. Glycine gave distinct increases in the blood urea values.

Compared with glycine, lysine is metabolized slowly, excreted readily in the urine if given in large quantities, and shows little, if any, specific dynamic action.

*Further studies on the arterial pulse.* PHILIP DOW (by invitation) and W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

The previously reported studies on the development of the peripheral pulse have been continued, using the high-frequency optical manometer and, as cannulae, either hypodermic needles or various lengths and sizes of needle tubing.

Many records of human blood pressure have been analyzed. The majority of the experiments have been done on intact dogs, in which the only necessary surgery was the exposure of the carotid and femoral arteries under morphine or morphine and ether anesthesia. A small rubber balloon at the tip of a long cannula permits occlusion at will of an artery at any point which can be reached with the cannula.

Emphasis has been placed on the abdominal aorta system, but the studies include analyses of the carotid and brachial systems as well. Attention has been directed to time relationships more than to pressures, and the parts played by transmitted waves, reflected waves, standing waves, and blood velocity have been evaluated.

*The effects of removal of vestibular parts of the cerebellum in primates.* R. S. Dow<sup>1</sup> (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Operative removal of the nodulus and lower part of the uvula in monkeys (*Macaca mulatta*) is followed by a syndrome of disequilibrium consisting of side to side oscillations of the head and trunk with falling forward, backward, and to either side. The animals exhibited a widely abducted posture and gait, and frequent titubations in walking and running were constantly present. They were totally unable to maintain their position upon a horizontal bar. Following this lesion they did not leap about an enclosure, a behavior characteristic of normal animals. This syndrome gradually disappeared during a period of from five to eight weeks. Throughout the post-operative period all the vestibular reflexes were present and there was a consistent absence of nystagmus. No sign of cerebellar ataxia was detected in the movements of the extremities. Excision of much larger portions of cerebellar cortex elsewhere in the vermis, including the pyramis, failed to produce this syndrome. A similar operative procedure, including manipulation of the nodulus and uvula within the fourth ventricle but without their removal, was not followed by any abnormality of posture or movement.

Anatomical findings show that the nodulus and lower part of the uvula have direct connection with the vestibular system. The disorder which follows a lesion here appears to be vestibular in nature. This syndrome was completely absent following the removal of nodulus and lower part of the uvula in a monkey which had recovered from bilateral destruction of the labyrinth. The function of the vestibular parts of the cerebellum and their relationship to the underlying vestibular reflexes is now being studied. The removal of the nodulus and uvula in a chimpanzee resulted in a similar but less severe and less persistent disability. The syndrome as seen in the chimpanzee resembles that often seen in children having a medullo-blastoma of the cerebellar vermis.

*The effect of the continuous intravenous injection of epinephrine on the blood pressure.* LESTER R. DRAGSTEDT, JOHN VAN PROHASKA (by invitation) and HERMAN P. HARMS (by invitation). Department of Surgery, University of Chicago, Chicago, Ill.

Within recent years surgeons have attempted to treat essential hypertension by operations designed to reduce the secretion of epinephrine, as by adrenal denervation or partial adrenalectomy. In the present work a study was made of the effect of the continuous intravenous administration of various doses of epinephrine on the blood pressure in twelve normal undrugged, unanesthetized dogs. The injection was successfully maintained for periods up to 15 days, and hypertension was likewise maintained for this time. Symptoms referable to inhibition of gastro-intestinal motility however developed and caused death of the majority of the animals within a shorter period. It is concluded that whereas a moderately long continued hypertension may be produced by the continuous injection of epinephrine, the amount required is sufficient to produce other specific systemic effects which are usually fatal.

<sup>1</sup> National Research Council Fellow.

*Motility of the large intestine of the ruminant.* H. H. DUKES and J. SAMPSON (by invitation). Department of Physiology, New York State Veterinary College, Cornell University, Ithaca, N. Y.

A study of gastrointestinal motility in the ruminant is in progress. Experiments have been made on some sixteen sheep. The animal is anesthetized, usually with chloral hydrate given intravenously, the abdomen is opened, and the subject transferred to a large tank containing physiological saline maintained at body temperature. In a few experiments the spinal cord was destroyed or the splanchnic nerves were sectioned, but the improvement in motility was not considerable except in the large intestine. On several occasions, instead of placing the animal in the saline bath, the open abdomen was filled with paraffin oil. Through the saline solution or the oil, motility was studied. Motion pictures have been made. This report deals with the motor activity of the large intestine.

The cecum shows peristalsis and antiperistalsis; several waves may be traveling at once. At times they are very powerful. The proximal part of the colon shows remarkable peristaltic action. Numerous powerful waves arise and travel toward the spiral (intermediate) part. The bowel may be pale and firm during their passage. Propulsion is plainly evident. The proximal colon also shows antiperistalsis. Peristaltic and antiperistaltic waves may neutralize each other. Antiperistalsis in the proximal colon and peristalsis in the cecum have been observed to occur alternately. The cecum and proximal colon pump ingesta into the spiral colon where pellet formation begins and dehydration is presumably active. The relatively small capacity and great motor activity of the cecum and proximal colon would prevent any considerable amount of bacterial action in them.

The spiral part of the colon shows peristalsis, rhythmic segmentations, and pendular or swaying movements. Many of the peristaltic waves appear to be continuations of waves in the proximal part. At times the swaying movements involve the entire spiral part. The distal part of the colon has as yet been studied very little.

Motility was illustrated with motion pictures.

*The glycogenolytic function of the liver in the absence of its assumed humoral and nervous control.* J. A. DYE. Department of Physiology, Cornell University, Ithaca, N. Y. (Read by title.)

It is quite generally thought that mobilization of liver glycogen is normally effected through sympathetic stimulation or through humoral means. The following results indicate that glucose mobilization from liver glycogen may occur in the absence of these well recognized mechanisms. The liver glycogen of fasting hypophysectomized cats falls to a vanishing point within 3 to 6 days after each of the following procedures: 1, bilateral splanchnectomy; 2, bilateral splanchnectomy with demedullation of the left adrenal and total removal of the right adrenal; 3, the same surgical procedure as in 2 plus section of the hepatic nerves; 4, left splanchnectomy with removal of the left lumbar sympathetics, section of the hepatic nerves, and total removal of the right adrenal, or 5, bilateral splanchnectomy, bilateral lumbar sympathectomy, section of the hepatic nerves, demedullation of the left adrenal, and total removal of the right adrenal.

Convulsive levels of hypoglycemia occurred as early, but not earlier, in these animals as in those subjected to hypophysectomy alone.

The denervated livers of these hypophysectomized-adrenal demedullated cats are also capable of storing glycogen. The livers of hypophysectomized cats which are in hypoglycemic convulsions or coma have glycogen values varying from 0.4 to 0.06 per cent. Intraperitoneal injections of glucose into these animals invariably lead to an increased liver glycogen—in one cat to 3.1 per cent.

It is concluded that the liver is able to respond to hypoglycemia by an increased glycogenolysis in the absence of its sympathetic nerve supply and after total removal of both the adrenal medullae and the hypophysis. Sympathin was not entirely excluded. The livers of hypophysectomized cats are unable to maintain blood sugar above convulsive hypoglycemic levels, at least in these denervated-adrenal demedullated animals, when the liver glycogen falls to about 0.4 per cent. The blood sugar level exerts a direct effect on the glycogenic function of this organ.

*The coagulation of blood by snake venoms and its physiologic significance.*

HARRY EAGLE. Department of Bacteriology, University of Pennsylvania, Philadelphia.

Nine of the 17 venoms here tested were found capable of coagulating citrated blood or plasma. As has been believed by most workers in the field, 7 of these 9 coagulant venoms convert fibrinogen to an insoluble modification resembling fibrin (*Bothrops atrox*, *Bothrops jararaca*, *Bothrops nummifera*, *Crotalus adamanteus*, *Crotalus horridus*, *Crotalus terrificus basiliscus*, *Crotalus terrificus terrificus*). The optimum pH for this coagulation was determined for 3 of these, and was found in each case to be approximately pH 6.5, the same as that for the action of thrombin on fibrinogen. Unlike thrombin, however, the fibrinogen-coagulating activity of the venoms was unaffected by the antithrombin elaborated in the course of anaphylactic shock.

In addition to coagulating fibrinogen directly, 3 of these venoms (*Bothrops atrox*, *Bothrops jararaca*, and to a less extent, *Crotalus terrificus basiliscus*) act on prothrombin to convert it to thrombin, without the necessary intervention of either calcium or platelets. Finally, there were 2 coagulant venoms in this series (*Notechis scutatus*, and to a slight extent, a mixed *Micrurus* venom), which had no demonstrable effect on purified fibrinogen, but which converted prothrombin to thrombin, and thus effected coagulation.

Unlike the reaction between the venoms and fibrinogen, this activation of prothrombin has no definite pH optimum, but takes place over a wide zone (pH 5.6–8.3). In the case of *Bothrops atrox*, there was some indication that the initial velocity of the reaction increased with increasing alkalinity, while the amount of thrombin ultimately formed decreased. Extraordinarily minute quantities of some of these venoms sufficed to produce a demonstrable activation of prothrombin. Thus, the fer de lance (*Bothrops atrox*) venom was active in a 1:25,000,000 dilution, and that of Australian tiger snake (*Notechis scutatus*) was active in a 1:4,000,000 dilution. The thrombin formed was indistinguishable from that produced by the action of calcium + platelets on prothrombin. Like the latter type of thrombin, and unlike venoms which act directly on fibrinogen, the venom-prothrombin mixture was inhibited by antithrombin.

Every one of the 9 non-coagulant venoms in this series destroyed prothrombin; and 5 of these destroyed fibrinogen as well.

As is discussed in the text, there is reason to believe that these several properties of the venoms (coagulation or destruction of fibrinogen; activation or destruction of prothrombin) depend on the proteolytic enzymes which they were found to contain. These observations lend further support to the thesis that, in the course of physiological coagulation, 1, calcium plus platelets (or tissue derivative) constitute an enzyme system which reacts with prothrombin to form thrombin, and which is thus analogous to trypsin and to several of the proteolytic venoms here discussed and 2, the thrombin so formed is itself a proteolytic enzyme which, like papain and the majority of the coagulant and proteolytic snake venoms here studied, reacts with fibrinogen to form a fibrillar gel, fibrin.

*The metabolism of lysine: the rate of absorption of d-lysine monohydrochloride from the gastro-intestinal tract in the rat.* A. G. EATON and J. R. DORY (by invitation). Department of Physiology, Louisiana State University Medical Center, New Orleans.

Adult rats were given approximately 500 mgm. of d-lysine monohydrochloride by stomach tube, and, according to the Cori technique, were killed and the alimentary tracts used for the determination of residual amino nitrogen. Estimation of amino nitrogen was made by the manometric method of Van Slyke. In some cases, after dilution of the gastrointestinal contents to a certain volume, aliquots were treated with phosphotungstic acid. The precipitates were then dissolved in dilute alkali and analyzed for amino nitrogen.

The absorption rates were calculated on the basis of the milligrams of d-lysine monohydrochloride absorbed per hour per 100 grams of rat. Results with and without phosphotungstic acid precipitation were not strikingly different. The average absorption rate for the two hour group, as measured by the total amino acid nitrogen, was 37.3, for the three hour group 27.8, and for the four hour group 21.0. When measured by the amino nitrogen precipitated by phosphotungstic acid the values averaged 34.7, 31.3 and 25.1 respectively for the two, three, and four hour periods.

These results show a definite decrease in absorption rate with increasing time as Wilson has demonstrated also for glycine and alanine. The average absorption rate is much less than that exhibited by glycine and alanine. It appears to be of the same order as that found for cystine.

*Stimulation of the hypothalamus in chronic hemidecorticated monkeys.*

LEON ECTORS<sup>1</sup> (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. (Read by title.)

Using the Horsley Clark stereotaxic apparatus, the hypothalamic region has been stimulated in normal and in hemidecorticated monkeys (*Macaca mulatta*). Faradic current and condenser discharges at a rate of 2 to 10 per second have been used for excitation. In normal monkeys tracts for pupil dilatation, increased blood pressure, increased respiration were followed from the supraoptic region down to the posterior end of the mesencephalon both in the median and lateral hypothalamus. In the anterior hypo-

<sup>1</sup> Fellow of the Committee on Relief of Belgium Educational Foundation.

thalamus the increased respiration was mainly an increase in amplitude; in the posterior hypothalamus it was chiefly an increase in frequency with long after-effect (panting). The stimulation of the posterior hypothalamus gave running and struggling movements.

Complete unilateral removal of the cortex was successfully carried out in eight monkeys. From one to two months after the operation, the hypothalamus of these animals was stimulated. The results obtained were the following.

1. The sympathetic responses (pupil dilatation, blood pressure rise, respiration increase) were identical from stimulation of either side and identical with the responses obtained in normal animals.

2. The motor movements (running, struggling) were obtained by stimulation of either side.

3. Stimulation of the tegmentum on both sides gave a typical tegmental response, stimulation of the pyramidal system on the decorticated side gave no response.

*Demonstrable functions of the renal tubule after it has been segmentally injured by the action of mercuric chloride.* J. GRAHAM EDWARDS. University of Buffalo, Buffalo, N. Y.

Segmental injury to the renal tubule of the frog and rat caused by mercuric chloride is manifest in the proximal convolution. The ascending limb of the medullary loop (rat) and the distal convolution are rarely injured. Functional evidence of lack of injury is observable under the microscope in the tubules of the living animal as follows. 1. Concentration. In the frog's kidney, dyes are seen concentrated in the lumen of the first half of the distal convolution. The degree of concentration, as observed, is very similar to that present in the lumen of this portion of the tubule in the kidney of the untreated frog when such dyes are being excreted. Certain iron salts appear similarly concentrated in this portion. Both dyes and iron salts are concentrated even when the entire proximal convolution is necrotic. In the rat, the lumen of the ascending limb and distal convolution contain concentrated dyes and iron salts when as much as two-thirds of the proximal convolution is necrotic.

2. Acidification. The presence of phenol red in the lumen of the distal half of the distal convolution (frog) and distal convolution in the rat is revealed by a lemon-yellow color and by a red color in the lumen of the preceding portion. The pH of such urine as may be excreted through these segmentally injured tubules is lower than normal as determined by using a potentiometer with a double quinhydrone electrode. Impairment of the intrinsic concentrating power of the cells of a certain portion of the tubule and that of the acidification of the urine by those of another, adjacent portion is thus not apparent in kidneys damaged by mercuric chloride poisoning.

*Purification of adrenal extracts and isolation of an activator of male sex hormones.* M. EHRENSTEIN (by invitation) and S. W. BRITTON. Physiological Laboratory, University of Virginia.

By a modified method cortico-adrenal extracts may be prepared which are uniformly high in potency. Fractionation procedures have given more refined extracts which possess greater physiological activity. Such relatively pure extracts contain up to twice the number of dog units per kilo

of fresh gland tissue and only one-half the amount of solid material present in the routine preparations.

A crystallized acid sodium salt of palmitic acid,  $C_{15}H_{31}COOH:C_{15}H_{31}COONa$ , has been isolated from one fraction derived from the adrenal cortex. Its importance as a very powerful activator of male sex hormones is discussed. Acid soaps other than that of palmitic acid were also obtained from the same fraction. Tests in combination with testosterone have been carried out on castrated young male rats.

*Observations on the coronary blood flow, electrocardiogram and blood pressure of the intact dog.* H. E. ESSEX, J. F. HERRICK, E. J. BALDES and F. C. MANN. The Mayo Foundation, Rochester, Minn.

Further observations have been made on the blood flow in the circumflex branch of the left coronary artery of the intact dog by use of the thermomuh. To determine the effect of the presence of the unit on the nutrition, conduction, rhythm, rate and efficiency of the heart electrocardiograms and blood pressures were taken during the same period. That the nutrition, conduction and rhythm of the heart were unimpaired was shown by the persistence of normal electrocardiograms for several days following the operation. Subsequent changes were comparable to those reported by Barnes and Mann following operations in which only the pericardium was incised. The efficiency of the heart is not impaired insofar as maintenance of normal blood pressure is concerned (Hamilton and Woodbury method). The rhythm of the heart is usually unaffected. The rate of the heart is usually increased. This is due in part at least to an increased body temperature which almost invariably develops postoperatively in the dog. The values for blood flow are comparable to those obtained in previous experiments. For example, the flow in the circumflex branch of the left coronary artery of an 18-kgm. dog was 58 to 62 cc. per minute on the fourth postoperative day. From the work done thus far we may conclude that the blood flow values obtained are a reasonably close approximation of what occurs in the normal animal.

*The lactogenic hormone of the anterior pituitary.* EVERETT IDRIS EVANS.

Department of Pharmacology, University of Chicago, Chicago, Ill.

Earlier work by Grüter and Stricker, Corner, and Riddle and Bates has demonstrated a lactogenic principle in the anterior pituitary gland. Riddle and Bates, in a later paper, presented data that indicated that the lactogenic substance might be a separate and distinct principle of the anterior lobe.

The author has prepared the lactogenic principle by a new method of extraction, precipitation, and separation from the gonadotropic and thyrotropic factors. The gist of this new method is as follows: Most of the procedures are carried out at 2 to 5° Centigrade. Beef anterior pituitary is extracted repeatedly with 0.05 N NaOH and these extracts are then neutralized with 0.05 N HCl. A very considerable portion of the inert protein is then precipitated out with Calcium salts, or other salts of the Ca, Ba, Sr series. The supernatant fluid contains most, if not all, of the lactogenic principle. This principle is then precipitated out at its isoelectric point, circa pH 5.0, by careful adjustment of the pH with 0.05 N HCl and with control of the salt concentration. Two other fractions may then be precipitated out at pH 4.3 and 3.2, respectively.

The first fraction contains 80 to 90 per cent of the lactogenic principle and this fraction assays 8 to 15 units per milligram; the second fraction contains 10 to 20 per cent of the lactogenic principle and this assays 1 to 2 units per milligram; the third fraction contains none of the lactogenic principle.

The first fraction may then be freed of the adsorbed gonadotropic principle by repeated precipitation at pH 5.0. It will be found then to contain no thyreotropic or gonadotropic factors. Further purification has been accomplished by use of fractional alcohol treatment.

Preparations have been made that assay as high as 33 units per milligram.

The new lactogenic preparation of the anterior pituitary will stimulate milk secretion in rabbits at 1 mgm. per kilo; lactation has been induced in six nulliparous and multiparous human females and one case of gynecomastia in the male, using doses from 1000 to 2250 units.

The lactogenic principle is apparently a protein substance, reacting strongly to the Biuret, Ninhydrin, Xanthoprotein, Hopkins-Cole and is negative to Koessler-Hanke and Millon reagents. There are indications that the present preparations are rich in tryptophane, but contain no tyrosine. There is a highly labile sulfur group in the impure preparations, but none in the purer preparations. There is about 3.5 per cent cystine in those preparations assaying 25 units per milligram. In all but one experiment, this new lactogenic preparation was destroyed by boiling for one hour at pH 7.8 to 8.2. This particular lactogenic preparation contains no vasodilator substances.

*The assay of the lactogenic hormone.* EVERETT IDRIS EVANS. Bureau of Dairy Industry, U. S. Dept. of Agriculture, Washington, D. C. (Read by title.)

Riddle and Bates have reported earlier that there is a quantitative relationship between the dose and crop gland response of immature pigeons when these birds are injected with lactogenic hormone. Other workers have had difficulty in using pigeons for quantitative assay of this hormone.

The author used two races of pigeons, White Carneaux and White Kings. The birds were 8 weeks of age, injections were made deep in the breast muscle daily for four days, and the crop glands were weighed on the fifth day. It was found that with the White Carneaux birds there was excellent logarithmic response with increasing dosage. This did not obtain with the White King race, there being great variability in response at a given dose level. It was concluded that these experiments confirm those of Riddle and Bates for the White Carneaux pigeons, and that these birds can be used for assay of the lactogenic hormone.

It was found, however, that there was no correlation between the dose and the weights of the thyroid and the testes of immature male pigeons injected with material containing the thyreotropic and gonadotropic factors; we concluded that these birds could only be used for a qualitative test for these factors, and not for quantitative assay of the thyreotropic and gonadotropic factors.

*"In the beginning."* EVERETT IDRIS EVANS. Bureau of Dairy Industry, U. S. Dept. of Agriculture, Washington, D. C. (Motion picture.)

This motion picture is intended to present in an artistic, yet factually correct manner, the details of the early development of the mammalian

ovum in domestic animals. It is hoped that it may be used by teachers of physiology and biology in elementary schools, as well as colleges and universities, as an introduction to the study of the physiology of reproduction. It is available as a rental without charge by application to the Division of Motion Pictures, U. S. Dept. of Agriculture, Washington, D. C. It is a sound motion picture, and is available only in the 35 mm. size.

*The origin of cochlear potentials.* J. A. E. EYSTER, T. H. BAST (by invitation) and M. R. KRASNO (by invitation). Departments of Physiology and Anatomy, University of Wisconsin Medical School, Madison.

In a series of observations on guinea pigs it is found that in this animal, the development of normal cochlear potential in response to air or bone conducted tones does not depend on the Organs of Corti, the tectorial or Reissner's membranes, or on an intact cochlear duct. The results also offer strong evidence against a nervous origin of these potentials. Passage of a strong polarizing current through the cochlea has no effect until evident electrolysis occurs, producing, as shown by subsequent histological examination, extensive disruption of membranes and gross physical damage. Various toxic materials applied to the membrane of the round window produce little or no effect except through damage to the middle ear. The same is true if these materials are introduced directly into the cochlea, provided no serious physical damage is done to this structure and middle ear effects are avoided.

The cochlear response appears to be extraordinarily resistant to all influences not causing gross physical change. It has been previously shown that disturbance of pressure relations between the endo and perilymph systems will greatly reduce or abolish the response. This observation, together with those reported at this time, lead us to the conclusion that the cochlear potentials have a purely physical origin. In a vibrating mechanism consisting of aqueous phases separated by membranes, such as the cochlea represents, it would appear that electrical potentials would inevitably result from the physical pattern present.

*Production of experimental diabetes insipidus in cats.* LEE E. FARR, KENDRICK HARE and R. A. PHILLIPS (introduced by J. C. Hinsey). Hospital of the Rockefeller Institute for Medical Research and the Department of Physiology, Cornell University Medical College, New York City.

Seven cats in which hypothalamic lesions were made through a subtemporal exposure developed permanent polyuria and polydipsia. The lesions, transverse ones made with the bent tip of a dissecting needle, were placed to interrupt the supraoptico-hypophyseal system at 1, the supraoptic nucleus; 2, in the ventral part of the tuber cinereum, or 3, in the pituitary stalk. Immediately following recovery from the anesthetic all the cats save one had an increased water intake and augmented excretion of very dilute urine, but within 72 hours this disturbance disappeared and the water exchange remained within normal limits until 13 to 22 days after the operation. At this time there began an increase in the volume of the urine, a decrease in its specific gravity and, less than 24 hours later, an increase in water consumption. These changes were gradual, attaining their maximum 24 to 35 days after the operation. Unoperated cats usually drink no water and never more than 50 cc. per day, and excrete

50 to 125 cc. of urine with a specific gravity of 1.028 to 1.050. The operated cats drink 250 to 1100 cc. of water and void 200 to 1100 cc. of urine having a specific gravity of 1.005 to 1.015. All the cats were maintained under the same conditions and fed the same diet.

The cats with diabetes insipidus were extremely sensitive to the diuretic action of intraperitoneal injections of saline suspensions of the anterior lobe of beef pituitary. Urea clearances have been determined on both normal and diabetic cats over a wide range of urine flows.

*The effects of anesthesia on dogs.*<sup>1</sup> *Ethyl ether.* MARION FAY (by invitation), ESTHER GREISHEIMER, ROBERTA HAFKESBRING and (by invitation) MARIE ANDERSCH, MARJORIE KENYON, WINONA MACCALMONT, MARGARET SHARPE and RUTH CORTELL. Woman's Medical College of Pennsylvania, Philadelphia.

This experiment was undertaken to establish values for blood constituents in dogs before, during and after ether anesthesia. The electrocardiogram, blood pressure, and temperature were also studied.

Blood was drawn by cardiac puncture at weekly intervals to establish normal levels for sugar, non-protein nitrogen, fibrinogen, albumin and globulin, carbon dioxide combining power, serum chloride, potassium, total base, lactic acid, inorganic phosphate, pH, fatty acids, cholesterol, phospholipid, hemoglobin, red, white and differential counts. These values were compared with those obtained after one hour of ether anesthesia. After several control periods, the anesthesia was repeated.

No significant differences were found for non-protein nitrogen, fibrinogen, albumin and globulin, serum chloride, total base, white and differential counts. Variations which were not strictly referable to ether were observed in fatty acids, cholesterol and phospholipid. Sugar, lactic acid, inorganic phosphate, hemoglobin and red corpuscles showed consistent increases, while serum potassium, pH level, carbon dioxide combining power, and the water content of both whole blood and serum decreased.

Moderate decreases in blood pressure and temperature occur under ether. There was a marked increase in heart rate and the sinus arrhythmia normally observed in dogs disappeared. In several cases ventricular premature beats appeared after cardiac puncture but these were transient.

*The formation of an acetylcholine-like substance by excised tissues.* J. F. FAZEKAS (by invitation) and H. E. HIMWICH. Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. (Read by title.)

Quastel has shown that a substance having all the properties of acetylcholine is formed on the addition of eserine to the fluid medium in which brain slices are suspended. This substance is hydrolyzed by alkalis and does not accumulate in the absence of eserine. Small amounts of this acetylcholine-like substance cause contraction of the leech muscle. Furthermore, Quastel has demonstrated that the oxidation of glucose added to the suspension of brain slices increases the rate of production of this acetylcholine-like substance. In all the above facts we have confirmed Quastel's observations with the exception that we could not, in every case,

<sup>1</sup> This work was aided by a grant from the Penrose Fund of the American Philosophical Society.

correlate the rate of glucose consumption with that of the production of this acetylcholine-like substance.

We have extended these observations to include the gastro-intestinal tract. Rat intestine sliced longitudinally, epithelial cells remaining intact, was permitted to respire with and without eserine in a Warburg respirometer. It was observed that in the absence of eserine no acetylcholine-like substance was produced as evidenced by failure of response of the leech muscle, while in the presence of eserine significant responses were obtained. Small intestine, per milligram of tissue, produces approximately one-half as much acetylcholine-like substance as does the brain. It is astonishing that this organ, which contains but a small proportion of nerve tissue, should be able to produce such comparatively large amounts of the acetylcholine-like substance. Further experimentation is in progress to quantitate more accurately the comparative rates of production of acetylcholine-like substance by these tissues and to determine the factors which influence its production.

*Factors affecting the loss of potassium from muscles on stimulation.* W. O. FENN. Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

The loss of potassium (per gram dry weight of muscle) in exchange for sodium as a result of indirect stimulation for 30 minutes has been confirmed in decerebrate and in anesthetized cats (Dial). Little or no change in potassium content of the blood is observed. The loss of potassium from muscle is greater if the muscle contracts isometrically than if the tendons are cut; it is greater if the circulation is good than when the animal is over-anesthetized or moribund. In one case a gain of potassium was observed in muscles which fatigued excessively rapidly after a long period of anesthesia. Stimulation causes generally a greater loss in decerebrate animals than in animals under Dial; the loss is greater soon after decerebration than if the stimulation is delayed a few hours. If the circulation to a stimulated muscle is partially depleted by periodic occlusion of the artery fatigue develops more rapidly and completely and the muscle gains more water, but the loss of potassium is less than in a well circulated muscle. A muscle which is continuously tetanized for 30 minutes loses less potassium than one tetanized for 0.5 second at 2-second intervals. The soleus muscle accumulates much less lactic acid in 30 minutes of stimulation than the other muscles of the lower leg and at the same time loses more potassium. Unlike the other muscles the soleus also loses more potassium on continuous than on rhythmic tetanization; presumably it has an unusually good circulation. During recovery, potassium returns to the muscle which lost it during stimulation. Rat muscles lose potassium after prolonged and vigorous exercise by swimming in a waterbath as compared with the denervated control muscles. Frog muscles can now be shown to lose potassium if stimulated for periods of one hour or more without excessive fatigue.

*The action of calcium in blood coagulation.* JOHN H. FERGUSON. Department of Physiology and Pharmacology, School of Medicine, University of Alabama, University.

Plasma prothrombin was activated to thrombin by the addition of cephalin and calcium salts (Ferguson: *Am. J. Physiol.* **117**: 587, 1936):

Citration (or oxalation) was effective in preventing this activation only if added within a minute of mixing the reagents. The process of thrombin formation was maximal in 6-7 minutes.

"Fresh" thrombin (i.e., about 10 minutes from time of mixing) could be progressively inactivated by incubation with a large excess of citrate or oxalate. "Ripe" thrombin (i.e., several hours old) showed no impairment of clotting power on oxalation or citration.

Electrodialyzed "ripe" thrombin (completely freed from calcium) gave excellent clots even in the presence of enormous excess of oxalate or citrate, provided that the pH was suitably adjusted prior to the addition of the prothrombin-free test fibrinogen. Incubation with citrate or oxalate had no effect upon the activity of electrodialyzed thrombin other than the slight "immediate effect" always noted in the presence of these anticoagulants (Ferguson: Proc. Soc. Exper. Biol. and Med. **34**: 797, 1936). Our thrombins were remarkably stable, retaining their activity for many days and even resisting boiling to some extent.

The present data reconcile the conflicting views in the literature and clearly indicate that calcium forms an *intermediary complex* (prothrombin + cephalin + calcium "compound"). The intermediary complex soon passes over into a stable thrombin. The intermediary can readily be deprived of its calcium, whereas the final coagulant can be prepared calcium-free without significant loss in potency.

*Carbonic anhydrase in marine invertebrates.* J. K. W. FERGUSON, LENA LEWIS (by invitation) and JUDITH SMITH (by invitation). The Marine Biological Laboratory, Woods Hole, Mass.

Robertson and Ferguson (Proc. Am. Physiol. Soc., 1936) reported certain features of the distribution of carbonic anhydrase in the tissues and body fluids of invertebrates. A quantitative comparison of the activity of the enzyme in various tissues was not attempted, because sufficient information as to the most effective methods of extracting the enzyme from tissues was not available.

The relative merits of several extracting agents have now been tested, including isotonic NaCl, glycerine, alkaline phosphate and H<sub>2</sub>O. The glycerine extracts were the most active but all solutions were found to be more efficient if the tissue were frozen and pulverized before extraction. With this procedure H<sub>2</sub>O extracts were almost as active as glycerine extracts and were used routinely. Twelve to eighteen hours seemed to be the optimal time of extraction.

The manometric method of Meldrum and Roughton (J. Physiol. **80**) was used to assay the extracts and the results are expressed in units (E) as defined by them.

The squid (*Loligo pealei*) is particularly suitable for studying the distribution of the enzyme among the tissues because the blood shows negligible activity. Extracts of gill tissue however were not far short of the potency exhibited by mammalian blood giving activities up to 1000 E per g. of original gill tissue. Extracts of other tissues showed much less activity; e.g., muscle gave 30-50 E/g; skin, 3 E/g; tentacles, 10 E/g; eggs, 100 E/g.

Other invertebrates including the lobster (*Homarus americanus*), the horseshoe crab (*Limulus polyphemus*), the spider crab (*Libinia emarginata*) showed the same preponderance of activity in the gills though the maxi-

mum activities were much less. For example, the gills of limulus gave up to 330 E/g; lobster gills up to 64 E/g and spider crab gills up to 43 E/g.

At first sight the situation of carbonic anhydrase in gills seems ideal to facilitate the escape of  $\text{CO}_2$  from the blood. It is difficult however to postulate any ionic exchange between the cells of the gill and the blood which would allow the enzyme to function that way if it is really intracellular.

*The time course of the frequency change when a carbon monoxide poisoned heart is illuminated.* KENNETH C. FISHER and J. A. CAMERON (introduced by Laurence Irving). Marine Biological Laboratory, Woods Hole, and the Department of Zoology, University of Maine, Orono.

When embryos of *Fundulus* are subjected to  $\text{CO}$  containing 3 per cent  $\text{O}_2$  the steady frequency of the heart becomes a function of the light intensity (I) according to the relation

$$\frac{\text{uninhibited frequency}}{\text{inhibited frequency}} = K \frac{\text{O}_2}{\text{CO}} I$$

With heart beat frequency replacing respiration this equation is a simplification of one derived originally by Warburg for the behavior of respiratory  $\text{O}_2$  uptake in the presence of  $\text{CO}$ .

Though the respiration of a  $\text{CO}$  poisoned tissue changes practically instantaneously to a new constant value upon illumination such a rapid change was not observed for the frequency of the heart. Consequently one wishes to determine whether the interval required for the change of frequency is the time for the photochemical decomposition of the  $\text{CO}$ -enzyme complex or whether it represents the utilization of some other store of reducible substance.

The time course of the frequency change (occasioned either by light or dark) in our data is accurately represented by the equation of a first order process. Comparison of the removal of inhibition due to illumination with the immediately subsequent redevelopment of inhibition in dim light has not revealed any simple relation between the velocities of the two. These do not appear to be necessarily equal though often are so.

Consideration of the curves obtained at different light intensities and preliminary observations at two different temperatures makes it improbable that the time course is that of a photochemical change. The simplest possibility, namely that the reserve implied by the inertia towards a change of frequency is oxidized ferment, is then not tenable. Indications as to the relation of the reserve to other known chemical systems are not yet available.

*Units of electrical activity in the cerebral cortex.* A. FORBES, B. RENSHAW (by invitation) and B. REMPEL (by invitation). Department of Physiology, Harvard Medical School, Boston, Mass.

Pentobarbital anesthesia approaching lethal depth is effective in isolating single action potentials derived from the surface of the cerebral cortex of the cat. Micro-electrodes consisting of glass pipettes drawn to a terminal aperture of from 15 to 100  $\mu$  and filled with Ringer solution, thrust into the gray matter of the cortex, still further segregate these action potentials.

Frequently repetition of simple monophasic excursions of about 40

msec. duration suggests physiological units—organized groups of neurones, or possibly single cells. These often show a regular rhythm varying between 5 and 7 per second. Sometimes negative and positive excursions appear in regular alternation. The deeper the anesthesia the simpler and more uniform is the picture, which suggests differential narcosis of different types of cell.

Changes in the pattern of response were regularly correlated with the depth of penetration of the electrodes. In some of these experiments the polarity of the majority of action potentials was reversed, the exploring electrode changing from positive to negative as it penetrated beyond a certain depth. This is consistent with Adrian's interpretation of the "deep response," although sometimes a change in duration suggested derivation from a different unit. In general the greatest source of potential appears to be between 0.5 mm. and 2 mm. below the surface.

Differences of potential between two micro-electrodes separated by only 0.1 to 0.2 mm. are about as large as those recorded with greater separation. Changes of position of like dimensions grossly alter the pattern. These results reveal sharply localized sources of potential difference. Two-channel recording occasionally shows approximately simultaneous waves in which two such micro-electrodes change potential in opposite directions relative to a distant common ground.

*Mechanical properties of nerve proteins in unimolecular films.* LYMAN FOURT (introduced by F. O. Schmitt). Department of Zoology, Washington University, St. Louis, Mo.

These studies were undertaken to obtain further information about the properties of the principal protein complex of the axon, neuronin, which might lead to a better understanding of the optical and mechanical properties of the living axis cylinder.

Since "structure" in a labile protein complex such as the axis cylinder depends on integration of the protein chains and micelles by interaction and lateral cohesion of the aggregates, a method has been devised to measure these forces quantitatively in unimolecular films. The method consists of the measurement of the period and damping of a vane oscillating in the surface, as a function of the age and degree of compression of the film and the nature of the subsolution on which the film is spread. The assumption is made that the damping is due to the rupture of the film at the ends of the vane. The mechanical strength is the force required to break through the film at the rate of one centimeter per second, and should be comparable to the tensile strength. Elasticity, which likewise results from lateral cohesion of the aggregates, is also measured and is found to vary with film conditions in general as does the mechanical strength.

Neuronin obtained by neutral aqueous extraction of lobster nerve spreads more or less rapidly, depending on the nature of the subsolution, to form either fluid or elastic films. A change with time from the fluid, non-coherent "sol" state to the elastic, tenacious "gel" state can be followed on certain subsolutions. This spontaneous change in state indicates that the changes in orientation and cohesion of the aggregates (through side chain reactions?) are effected by chemical readjustments following spreading as well as by increased compression. The maximum mechanical strength measured is one tenth to one hundredth that of textile fibers.

The effect of the chemical environment (composition of the subsolution)

on the properties of the film is discussed as an indication of the type of linkage responsible for the structure of the film.

*An ovarian factor, other than progesterone, which inhibits the uterine response to estrin.* S. C. FREED (by invitation) and SAMUEL SOSKIN. Department of Metabolism and Endocrinology, Michael Reese Hospital, Chicago, Ill.

We have recently shown that the uterus of the castrate rat responds much more promptly to the administration of estrogenic substance, than does the uterus of the normal animal. We have ascribed this difference to the presence of an estrus-inhibiting factor in the rat's ovaries. This is strikingly demonstrated by the following results.

Each of 16 adult female rats was injected with 250 international units of dihydroxyestrin benzoate per day, for 3 days. On the fourth day the animals were anesthetized and their abdomens opened. In 11 of the animals the uterus was in diestrus, in 2 there was evidence of early estrus, and in only 3 was there definite estrus. This distribution of results is similar to that found upon opening a corresponding group of normal, untreated adult female rats. We have previously shown that the administration of estrin for as long as 20 days shows a similar lack of effect. After recording the state of the uteri, 10 of the animals were ovariectomized and the abdomens of all were sutured. Three days later, without any further treatment, all the animals were sacrificed for examination. In every one of the 10 castrated animals the uterus had developed a typical estrus reaction. The uteri in the 6 control animals were essentially unchanged.

It is evident that ovariectomy removed an inhibiting influence, which had prevented the response to estrin. This inhibition cannot be ascribed to progesterone, since the simultaneous administration of progesterone with estrin to castrate females either fails to inhibit the estrus (small amounts of progesterone) or results in a uterus resembling that of the pregnant animal (large amounts of progesterone). It is, therefore, concluded that there is a factor in the ovary other than progesterone which inhibits the action of estrin on the uterus. Preliminary experiments indicate that this inhibiting factor arises from the corpus luteum.

*The effect of temperature on the volume flow of blood through the sympathectomized paw of the dog, with observations on the oxygen content and capacity, carbon-dioxide content and pH of the arterial and venous blood.* NORMAN E. FREEMAN and J. WALLACE ZELLER (by invitation). Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital, Boston.

Plethysmographic determinations of the volume flow of blood through the sympathectomized paws of three unanesthetized, trained dogs, in which one adrenal was removed and the other denervated, showed that the circulation varied directly with the temperature of the bath in which the paw was immersed. The oxygen content, carbon-dioxide content and pH of the arterial and venous blood were constant within the limits of experimental error over wide ranges of temperature and blood flow. These observations are consistent with the hypothesis that the circulation through regions deprived of vasomotor control is determined by the metabolic needs of the tissues.

*The effect of jaundiced blood or serum upon the serum phosphatase, inorganic phosphorus, and sugar of normal dogs.* SMITH FREEMAN and Y. P.

CHEN (introduced by F. T. Jung). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

A series of blood transfusions and serum injections have been carried out on dogs; the donors have been dogs whose common bile ducts were ligated from 6 to 21 days previously and the recipients have all been normal dogs. The serum phosphatase, inorganic phosphorus and sugar have been determined at intervals before and after such transfusions. The serum phosphatase of the recipient is invariably elevated by fifteen or twenty units per 100 cc. of serum; the elevation may persist for as long as 150 hours and its decline is always quite gradual. The inorganic phosphorus of the blood serum changes in one of two directions depending upon the serum sugar response to the transfusion; if the sugar of the serum is elevated the inorganic phosphorus decreases and if the serum sugar is unaltered the inorganic phosphorus is elevated. An attempt is being made to determine whether the changes in phosphorus and sugar are due to the elevation of the phosphatase in the blood stream. Active preparations of kidney phosphatase do not circulate for more than a few minutes when injected into the circulation.

*The effect of adrenalectomy upon experimental aluvotion and luteinization in anestrus cats.* H. B. FRIEDGOOD and M. A. FOSTER (by invitation). Department of Physiology, Harvard Medical School, and the Biological Laboratories, Harvard University, Boston, Mass.

Previous studies by Foster and Hisaw have demonstrated that the ovaries of anestrus cats can be stimulated experimentally by the injection of anterior hypophyseal gonadotropic hormones (FSH subcutaneously for 5 days followed by the intravenous administration of FSH plus LH). This stimulation results in a growth of follicles which subsequently ovulate and become luteinized normally. The present investigation is based upon the experimental observation of 20 normal anestrus cats which were treated in a similar manner. Ten of these animals were reserved for controls, and 10 were adrenalectomized at various intervals prior to the injection of the hormones. The animals in both series were sacrificed 24 to 120 hours after the final intravenous injection. The ovaries were studied macroscopically and cytologically; and the uteri were subjected to motility tests, viz., the adrenalin reversal reaction which indicates functional luteinization of ovulated follicles.

*Results.* The ovaries of each animal in both experimental groups contained from 15 to 25 ovulation points. The normal process of ovulation and subsequent luteinization was retarded in the adrenalectomized cats. This was determined by histological examination of the dehiscent ova and of the ruptured follicles and physiological studies of uterine motility. The adrenalin reversal reaction in the adrenalectomized cats occurred from 12 to 22 hours later than in the normal controls.

*The influence of filling the stomach on colon motility in the dog.* E. A. GALAPEAUX (by invitation) and R. D. TEMPLETON. Departments of Physiology, The University of Chicago and Loyola University School of Medicine, Chicago, Ill.

The term "feeding reflex" has been offered by Welch and Plant (Am. J. Med. Sci. 172: 261, 1926) as a substitute for the more commonly used term "gastro-colic reflex." Their suggestion is based upon experiments

in which no increase in colon motility was observed associated with filling the stomach by way of a gastrostomy, but an increase was observed when food was eaten.

Our investigations were conducted on trained dogs on which cecostomies had been performed several months prior to this study. The tandem balloon technique was employed for recording colon activity. One system of three balloons was inserted by way of the cecostomy and another set of three by way of the anus. Records of 400 minutes duration were obtained on a smoked paper. At the close of the first 200 minutes a fluid mass of 1100 cc. of buttermilk and one-half pound of yeast was introduced into the stomach by way of a stomach tube. No interruption in the tracing was made during the administration of the mixture.

A comparison of the quantity of activity in the second 200 minutes to that obtained in the first 200 minutes revealed an augmentation beginning shortly after the stomach was filled and increasing progressively to the end of the tracing.

These experiments are not concerned with the existence of a "feeding reflex," but present evidence that the term cannot be substituted, as suggested by Welch and Plant, for the term "gastro-colic reflex."

*Essential anatomical structures of the reflex arc for establishment of conditioned reflexes.* W. HORSLEY GANTT. Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md.

The classical unconditioned reflex arc consists of sense organ plus afferent nerve, central connection, efferent nerve plus executor organ. Ordinarily the conditioned reflex is built upon this by establishing functional connection between a stimulus falling upon sense organ and the central excitation produced by the unconditioned stimulus. Which of these elements are indispensable?

Executor organ and peripheral nerve have been eliminated without destroying the ability to form conditioned reflexes (experiments with Light elaborating conditioned reflexes in leg paralyzed by crushing all anterior nerve roots—conditioned withdrawal to signal appeared after nerve regeneration as result of training only during period paralysis).

Further experiments have eliminated the afferent limbs of both unconditioned and conditioned reflex. 1. Unconditioned reflexes were obtained after eliminating successively parts of the afferent arc, up to the motor area of the cortex, as follows: electrodes placed on the lumbar posterior roots in a chronic preparation produced an ipsilateral hind leg jerk, involving four legs with stronger current. This movement was conditioned to a bell. In other animals the afferent arc was successively shortened by placing the stimulating electrodes at the following levels: posterior spinal columns, within the cerebellar nuclei causing contraction of the ipsilateral leg (see abstract with Brogden), on motor area of cerebral cortex producing a leg movement (experiments of Loucks). In all but the last instance the movement evoked by direct faradization could be conditioned to signals. When electrodes are placed directly on the cortical motor region the existence of a reflex is questionable because the entire afferent limb has been shortcircuited. 2. The external receptor and the afferent nerve of the conditioned reflex arc were eliminated by replacing the usual conditioned signals by stimulations originating in various internal structures, viz., spinal cord, cerebellum, cortical sensory areas (area striata). Al-

though current spread to distant centers was unlikely, irradiation of the nervous impulse to activate other elements was possible.

Thus neither motor nor sensory periferal structures are necessary for conditioned reflex formation.

*Action of acetylcholine on cultures of chick heart.* W. E. GARREY. Physiology Department, Vanderbilt University School of Medicine, Nashville, Tenn. (Read by title.)

Preliminary experiments indicate that beating heart muscle of the chick developing in tissue cultures and therefore free from vagal inhibitory influences are not inhibited by acetylcholine in any physiological concentrations, even when eserine is present. Depression will occur with what may be considered toxic concentrations eg. one in 3000; the effects of these concentrations are comparable to those obtained by other workers on hearts of developing chicks prior to innervation and indicate that heart muscle itself, *sans nerfs*, is immune to the true inhibitory action of acetylcholine, thus confirming conclusions reached in experiments with the turtle ventricle.

*Effects of acetylcholine on the frog's ventricle.* W. E. GARREY and GORDON BASS (by invitation). Physiology Department, Vanderbilt University School of Medicine, Nashville, Tenn. (Read by title.)

The effects of acetylcholine have been determined on the basal, middle and apical thirds of the ventricles of three species of American frog: the common grass frog (*Rana pipiens* or *sphenocephala*), bull frog (*R. grylio*), and jumbo bull frog (*R. catesbeiana*). All portions of the ventricle were inhibited, and equally so far as could be ascertained by determining the minimal inotropically effective concentration, which in general was about one part in 25 million, (auricular effects are obtained with lower concentrations) and by noting that equal concentrations produced an equal percentage decrease in the height of contraction of the pieces of a given ventricle. This acetylcholine test for vagus innervation demonstrates the presence of vagus terminations in the apical portion of the ventricle of these frogs a result at variance with that reported by Láncoz for the European frog (*R. esculenta*).

*Acetylcholine action on the turtle heart.* W. E. GARREY and L. L. CHASTAIN (by invitation). Physiology Department, Vanderbilt University School of Medicine, Nashville, Tenn. (Read by title.)

Confirming our report to the Circulation Section in 1936, acetylcholine and related choline derivatives are without inhibitory effects on the ventricular muscle of the turtle heart which is devoid of all direct vagus influences. There has been no exception in over 400 experiments on the hearts of nine species of turtle. The concentrations of acetylcholine used ranged from one part in 500 million to one in 200 parts of Ringer's solution. The addition or treatment with eserine or prostigmine did not alter the experimental result whether the ventricle or ventricular strips were suspended in the (oxygenated) solution or were perfused. In the case of hearts *in situ* in the body eserine and acetylcholine were introduced into the blood or the blood replaced by similarly treated Ringer's solution without altering the excitability or contractility of the ventricle although the sinus and auricles were completely inhibited. Potassium chloride

added to the Ringer's solution until the contractions were reduced to one half the normal height did not institute any susceptibility of ventricular muscle to acetylcholine. In contrast to these findings the musculature of the sinus and auricles show extreme sensitiveness to inhibition by acetylcholine; when suspended in oxygenated Ringer's solution one part of acetylcholine in 500 million often weakened contractions, an effect which ensued quite uniformly when the solution contained one part in 50 million. The inotropic effects of vagus stimulation can be summed algebraically with those of acetylcholine on the sinus and auricle whereas ventricular muscle is entirely unaffected. We conclude that chelonian ventricular muscle *per se* is absolutely insensitive to the inhibitory action of acetylcholine and that inhibitory effects depend upon the fact that the heart muscle is (or has been) innervated by inhibitory nerves. The reaction to acetylcholine thus becomes a test of appropriate innervation. The results are in accord with those of Armstrong on developing fish hearts. Indications are that the mammalian ventricle comports itself like that of the turtle.

*Inotropic and chronotropic effects of acetylcholine upon the chelonian heart.*

W. E. GARREY and L. L. CHASTAIN (by invitation). Physiology Department, Vanderbilt University School of Medicine. (Read by title.)

In conformity with the quite general presentment in tracings published by other authors, several hundred experiments have shown us that inhibitory inotropic effects upon heart muscle are produced invariably by solutions of acetylcholine more dilute than those required to produce chronotropic effects. Obviously it is essential to make observation on muscle contracting in response to its own inherent rhythm. For this reason the isolated sinus and isolated auricles of the dogfish, frog and turtle and the beating isolated ventricle of the frog have been studied independently. In all instances dilutions of acetylcholine were found which caused weakening of contractions, as determined by mechanograms, electrograms, or both, without coincident changes in rate. Chronotropic depression required stronger solutions of acetylcholine for its superposition. All changes in rate were accompanied by significant weakening of contraction of the rhythmogenic muscle.

If one admits that inhibitory vagus effects upon both rate and strength of contraction are conditioned by the formation of acetylcholine (A Ch) and that the rhythm is inherent in the muscle, the conclusion is inevitable that all vagus fibers which produce chronotropic effects are also inotropic fibers. Many, if not all the divergent results thus far reported are due to a failure to consider the discrete topographic distribution of inhibitory nerves to the heart (Garrey).

*Responses of nerve to two trains of rhythmic stimuli.* H. S. GASSER. The Rockefeller Institute for Medical Research, New York City.

If a mammalian nerve is stimulated with a train of near-threshold shocks 50 msec. apart and a second train of stronger shocks at the same frequency is applied intercurrently at another electrode, so that the shocks of the latter fall half-way between the shocks of the former, most of the fibers start to follow the second series and cease responding to the first. The strong shocks of the second series can start a new train of responses despite the subnormal periods of the first train, while the weak shocks of the first series are ineffective during the subnormal periods of the second train.

The exclusion of one train by the other is most striking if the subnormality is exaggerated by rendering the nerve somewhat more alkaline than normal. If the nerve is acid (and the second series is made up of weak shocks), the two series through their supernormal periods facilitate each other.

The taking over of the responses from one train by the other offers a suggestion concerning the possible mechanism of reciprocal innervation. Transmission in the nervous system is effected by local summation of impulses at synapses. Let us consider an internuncial neurone common to both a flexor and an extensor arc, but receiving more nerve endings from the latter. During flexion this neurone would discharge simultaneously with other neurones on flexor motor neurones; but during extension it would be taken over into the group of internuncial neurones discharging upon extensor motor neurones, because of the dominant effect of the endings derived from the extensor arcs. The simultaneous discharge on the flexor motor neurones would be ineffective because out of time with the flexor circuits (impulses must arrive at intervals shorter than 0.5 msec. for summation to occur (Lorente de N6)); and the possibility of synchronization of the neurone with the flexor circuits would be lost, owing to the high threshold acquired from the stimulation in service of the extensor reflex.

*On the mechanism by which CO<sub>2</sub> offsets the effect of O<sub>2</sub> deficiency.* E. GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago.

In previous experiments (Gellhorn, *Nature* **137**: 700, 1936; *Am. J. Physiol.* **117**: 75, 1936) it was found that 3 per cent CO<sub>2</sub> offsets the effect of 8 to 8½ per cent O<sub>2</sub> in man in regard to various cortical processes.

To elucidate the mechanism involved blood pressure and temperature studies were carried out in man and animals. Significant differences in the blood pressure under conditions of O<sub>2</sub> deficiency with and without CO<sub>2</sub> were obtained in erect posture in the human.

Under O<sub>2</sub> deficiency the systolic blood pressure shows a temporary rise, which is followed by a fall which may eventually lead to collapse. In the presence of CO<sub>2</sub> this is prevented and the blood pressure remains elevated throughout the whole period of O<sub>2</sub> want. An acapnia similar in degree to that observed in experiments with O<sub>2</sub> deficiency leads to a lesser decrease of blood pressure under conditions of normal oxygenation than when an O<sub>2</sub> deficient gas is inhaled. From this it follows that CO<sub>2</sub> is more potent at a deficient oxygenation of the tissues than at a normal one. This conclusion is supported by blood pressure studies in animals in which it is found that the rise of blood pressure caused by O<sub>2</sub> deficiency is greatly augmented by small amounts of CO<sub>2</sub> which in themselves have no effect upon blood pressure. This reaction is present even under conditions of artificial respiration.

Furthermore, it is found that the fall in body temperature of rats exposed to low O<sub>2</sub> tension in the air is greater in the presence of 3 per cent CO<sub>2</sub> than in its absence. The restoration of circulation and the decreased O<sub>2</sub> demands of the tissues by the lowering of the body temperature are considered to be essential regulatory adjustments by which CO<sub>2</sub> offsets O<sub>2</sub> deficiency.

*The reversible inactivation of glycolysis by mercury compounds.* C. L. GEMMILL and L. HELLERMAN (by invitation). Johns Hopkins University, School of Medicine, Baltimore, Md.

In small concentrations, phenylmercuric chloride, p-chloromercuribenzoic acid and mercuric chloride inhibit glycolysis in extracts of frogs' muscle. This inhibition is reversed by the addition of cysteine or glutathione. A relationship exists between the amount of mercury compound present and the degree of inhibition of the glycolysis. A block in the glycolytic mechanism probably occurs at the hexose stage.

*The recruitment of inspiratory and expiratory mechanical energy in hyperpnea produced by the administration of sodium cyanide.* ROBERT GESELL and FLORENCE WHITE (by invitation). University of Michigan, Ann Arbor.

This subject was studied to determine the degree of reciprocal grading of activation of antagonistic respiratory muscles. Action potentials of the important respiratory muscles provided the index of intensity of contractions.

Intravenous injection of NaCN sufficient to increase pulmonary ventilation 4- to 6-fold produced varying results. Increase of inspiratory contractions predominated. In some individuals only increased inspiratory activity occurred unless the administration of NaCN was excessive. Even then activation of the expiratory muscles was weak and in special cases missing. In contrast to these findings occasional instances of equal excitation of expiratory muscles occurred. The duration of augmentation of expiratory contractions was decidedly shorter than that of the inspiratory muscles, beginning later and ending earlier. If the inspiratory or expiratory muscles were contracting upon a background of continuous activity cyanide abolished the continuous element between rhythmic contractions thus conserving mechanical energy. Conversely a common late effect of cyanide was the appearance of a relatively intense continuous background in the inspiratory muscles. When that occurred tonic expiratory contractions exhibited a reciprocal diminution only to increase again when the post administrative continuous background of contraction in the inspiratory muscles finally disappeared. A strong and rapid injection of CN properly timed powerfully augmented an inspiratory act already in progress. If properly timed during the expiratory pause it initiated an active expiration. From this it is temporarily inferred that both the expiratory and inspiratory mechanisms are independently susceptible to chemical stimulation.

*Conclusions.* Reciprocal grading of inspiratory and expiratory phasic contraction is a poorly developed integration of the central respiratory mechanism of the dog. The inspiratory side of this mechanism is more accessible to chemical stimulation than is the expiratory side. Respiration is largely an inspiratory act which provides expiratory energy by mechanical stretching of the torso and lungs, even during moderate hyperpnea. When purely inspiratory it approaches in function the simple pulsatile characteristics of the heart.

*Regulation of frequency in the cerebral cortex.* FREDERIC A. GIBBS. Harvard Medical School and Neurological Unit, Boston City Hospital, Boston, Mass.

The frequency of the fluctuations in potential which occur in the cerebral cortex is controlled, as is the frequency of respiratory movements and of heart rate, by the antagonistic action of rate accelerators and rate depressors. In general, the factors which tend to increase the rate of the respiratory center, as judged by increased frequency of respiratory movements, also increase the frequency of cortical potential fluctuations. In general, factors which tend to decrease the frequency of respiratory movements tend to decrease the frequency of cortical potentials. Except for time scale, there is a marked similarity between a record of respiratory movements and of cortical potentials made under similar conditions. Not only respiratory muscles, but all skeletal muscles record potential fluctuations in the central nervous system.

Measures which correct disturbances of respiratory rate tend to correct and prevent the comparable disturbances in cortical frequency which occur in epilepsy. Conditions which cause sudden changes in rate precipitate epileptic seizures. Because certain cell masses tend to "beat" at a characteristic frequency, frequency can have localizing significance in the nervous system. Slower frequencies modulate faster frequencies. The brain presents possibilities for almost infinite modulation.

*A unified concept of the mode of vagal cardio-inhibition.* A. S. GILSON, JR.  
Department of Physiology, Washington University School of Medicine,  
St. Louis, Mo.

The functional manifestations of vagal inhibition of the heart are such as would appear if critical recovery processes were slowed by the vagal action. An extension of this concept assumes that vagal effect results in the inactivation of a substance, the concentration of which determines the rate of recovery from a preceding discharge. The following types of evidence are among those which have been examined and found consistent with the hypothesis.

a. Chronotropic effect. If, following a previous pacemaker discharge, there is required time for recovery to a certain definite level before the next discharge, vagal effect would slow this recovery. Premature stimulation of the pacemaker is followed by an interval approximately or exactly equal to one uninterrupted cycle interval whether the latter be of normal length or prolonged by vagal inhibition. The hyperbolic relationship described by Rosenblueth as existing between vagus stimulus frequency and depression of heart rate indicates a simple chemical combination. The time course of chronotropic effect following a single vagus volley plots curves agreeing with empirical curves plotted under the theory.

b. Inotropic effect. (Here and below, a constant heart rate is assumed for simplicity.) Stimulation of the atrium (per ex.) by an impulse from the sinus causes a response of the atrium when the latter is incompletely recovered. There results a subnormal mechanical and electrical response. A corresponding argument applies to sinus or ventricular inotropic effects when these occur.

c. Bathmotropic effect. Prolonged relative refractoriness results in elevation of measured threshold.

d. Dromotropic effect. Seen outstandingly at junctional regions. Most severe when there is inhibition of both proximal and distal elements involved. There may be block in consequence of inhibition of only one element, block being due either to decreased impulse strength of the proximal element or to increased threshold of the distal element.

*A comparative study of the effects of histamine, acetyl- $\beta$ -methylcholine, and atropine on gastric secretion.* JOHN GRAY (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago, Ill.

Although atropine in dogs has been shown to completely abolish the gastric secretory response to a meal temporarily, no such striking action has been demonstrable against histamine secretion. We have therefore investigated the effect of atropine on histamine secretion, using the technique for quantitating gastric inhibition which has been recently developed in this laboratory. It was found that when a standard uniform level of gastric secretion (5 mgm. HCl per minute) is maintained in dogs with a pouch of the entire stomach by injecting histamine every ten minutes, atropine in 1 mg. doses is able to exert a definite inhibition of HCl production amounting to 30 or 50 per cent during the two hours following its subcutaneous injection. Two milligram doses show very little additional inhibition. As would be expected, the degree of inhibition is inversely related to the rate of secretion previous to the injection.

Since psychic secretion is presumably mediated by acetylcholine liberated in the gastric glands, the effect of acetylcholine and mecholyl (acetyl- $\beta$ -methyl choline) on gastric secretion were also investigated in total pouch dogs. It was found that small doses (less than 0.1 mgm.) of acetylcholine and mecholyl are more potent than histamine in stimulating the secretion of acid gastric juice when injected every ten minutes. However, in contrast to histamine, mecholyl secretion gradually declines after the second hour of injections; the decline being more rapid and complete with higher dosages. If the decline becomes nearly complete, the gastric glands become unresponsive to histamine. Large doses of mecholyl (1 mgm.) stimulate the production of a small quantity of an alkaline secretion high in mucus. Both of these actions of mecholyl can be demonstrated in dogs in which the uniform standard rate of secretion is maintained by repeated histamine injection. Small doses (0.1 mgm.) greatly increase the rate of acid production, whereas large doses (1.0 mgm.) inhibit acid secretion by 50 to 70 per cent. The excitatory as well as the inhibitory effects of mecholyl are completely abolished by 1 mgm. of atropine.

*Shock disease of wild snowshoe rabbits.* R. G. GREEN and C. L. LARSON (introduced by M. B. Visscher). University of Minnesota and Bureau of Biological Survey, Minneapolis.

Snowshoe hares throughout their range show a marked fluctuation in numbers at approximately ten-year periods. During the past three years snowshoe hares in Minnesota have been in their cyclic decline. We have recently found that hares taken from the wild are suffering from a predisposition to develop hypoglycemic shock and to die within a few hours after the first appearance of symptoms. During the spring of 1936, hares live-trapped in several places in Minnesota and held in captivity suffered a mortality of over 50 per cent by the fifth day of captivity and close to 100 per cent by the twentieth day. Blood sugars were found to be normal until symptoms appeared, at which time levels were found to be from 0 to 45 mgm. per 100 cc. Urinalysis performed on sick animals failed to reveal the presence of sugar in the urine. The symptoms developed are related to a decreased liver glycogen content which precedes the occurrence of shock conditions. The liver glycogen content in hares about to precipitate into shock averaged 0.14 per cent, and for hares already in shock was

usually less than 0.1 per cent. Glycogen values for normal hares have been found to vary from 2 to 5 per cent, with an occasional value as high as 12 per cent. Glucose solutions relieve the symptoms of shock in many cases. Adrenalin does not have this effect. Repeated injections of glucose solutions were effective for a time in alleviating shock symptoms, but eventually they failed to afford relief. An occasional hare would die atypically with a blood sugar of 250 mg. or over, although these animals had the usual low liver glycogen characteristic of the disease. Pathological studies showed that the essential lesion was a degenerative condition of the liver cells.

*Coronary responses associated with voluntary neuro-muscular activity.*

CHAS. W. GREENE. Department of Physiology, University of Missouri, Columbia.

Vascular reflexes associated with voluntary activity are unusually difficult of determination for the coronary blood vessels. By the electrical resistance method developed in Mann's Laboratory any increase or decrease in the flow of the coronaries is beautifully demonstrated under conditions of voluntary activity. The method which I have followed permits a further analysis of the factors contributing to the variation in coronary flow.

The method has a handicap in the difficulty of establishing voluntary muscular movements, since in experiments with the open chest the animal is under control of an anesthetic. When the anesthetic is so light as to establish semi-reflex movements of the skeletal muscular system, complicated difficulties in recording coronary changes develop with the lightness of the anesthetic and the increase in degree of voluntary control.

The preliminary experiments here reported are based on voluntary movements developed under acute recovery from surgical anesthesia in which the voluntary reactions are allowed to persist for only a short time. The data permits an analysis of the coronary reactions in association with 1, pronounced variation in cardiac response, obviously reflex in character, 2, a great increase in the volume of the respiratory movements, and 3, definite and independent fluctuation in coronary flow.

Analysis of experimental data shows that under these conditions the coronary variation is primarily a vascular reflex. Secondary influences may contribute, as for example the influence of the heart rate and volume and the changes in general blood pressure.

Furthermore, the coronary reflex is a primary coronary dilation. In the particular experiment illustrated by lantern slides and analytical data, the coronary dilation amounts to 70 per cent.

*The effect of changes in the electrolyte balance on the volume of plasma and extracellular fluid.*<sup>1</sup> MAGNUS I. GREGERSEN and GEORGE W. THORN (by invitation). University of Maryland School of Medicine and Chemical Division of the Medical Clinic, Johns Hopkins University, School of Medicine, Baltimore.

Alterations in the electrolyte balance unaccompanied by significant changes in the total body water were produced in dogs by intraperitoneal injection and subsequent withdrawal (2 hours) of large quantities (80 cc.

<sup>1</sup> Aided by grants (to M. I. Gregersen) from the Committee on Grants-in-Aid, National Research Council and The Rockefeller Foundation.

per kgm. body weight) of isotonic glucose (Darrow and Yannet, 1935), and by the intravenous injection of 30 per cent sodium chloride. The plasma volume was determined with the blue dye T-1824 (Gregersen, Gibson and Stead, 1935) and the "available fluid" volume with sodium thiocyanate (Crandall and Anderson, 1934; Lavietes, Bourdillon and Klinghoffer, 1936). The serum concentrations of both substances were measured spectrophotometrically (Gregersen and Stewart, to be published). The changes in the volume of plasma and total extracellular fluid were calculated from the plasma levels of T-1824 and thiocyanate, corrections being made for "disappearance" of the dye and removal of thiocyanate in the peritoneal fluid or urine. Plasma, peritoneal fluid, and urine were analyzed for sodium, potassium and chloride.

In the "glucose" experiments, about one-half of the decrease in total extracellular fluid (10 to 15 per cent) can be accounted for by the decrease in plasma volume (30 per cent or more). That the thiocyanate concentration actually reflects the changes in volume of extracellular fluid is indicated by the agreement between the amount of base removed from the peritoneal fluid and the decrease in total extracellular base, the latter being calculated from the plasma concentrations of base and the volumes of extracellular fluid before and after disturbance of the electrolyte balance.

*An optical blood pressure manometer.* DONALD E. GREGG. Western Reserve University, School of Medicine, Cleveland, O. (Demonstration.)

A small hypodermic optical blood pressure manometer is demonstrated. While it retains the principle of the Hamilton type of manometer (namely, a large effective mass combined with a very high volume-elasticity coefficient) certain changes have been made which aid greatly in the recording and analysis of curves. To insure greater ease in mechanical manipulation, a special manometer carriage has been devised, based on the principle of three point suspension, for rapid vertical and horizontal separate adjustments of the recording and base line in mirrors. The manometer is made of Monel metal or of transparent non-shatterable material to facilitate inspection for possible trapped bubbles. The recording membrane is generally of rubber although metal membranes are also used as an integral part of the manometer by machining down the manometer barrel end to the desired thickness. Rubber has the advantage that it gives good sensitivity (large curves) without long distance projection and without sacrificing the efficiency of the system. Individual, low dioptré, plano-convex mirrors are used. These are circular in shape and accurately ground to minimize prismatic effects and difficulties in matching focal lengths.

By a system of reflecting mirrors different manometers are tuned to the same sensitivity which largely eliminates the necessity for different calibration curves and at the same time permits long distance projection with the camera fairly close to the manometer.

*The phasic coronary blood flow in hypotension.* DONALD E. GREGG. Western Reserve University, School of Medicine, Cleveland, O.

The phasic blood flow in the ramus descendens anterior has been studied in hypodynamic hearts (aortic systolic pressures of 40-70 mm. Hg and diastolics of 10-40 mm. Hg) utilizing the method of differential pressure curves.

The velocity curve and the contour and time relations of the peripheral

coronary pressure curve are both similar to those previously described for the same coronary vessel under normal dynamic conditions but the range of diastolic and systolic coronary resistances, 8 to 15 and 30 to 50 mm. Hg respectively, are much lower. The blood flow is so fractionated that the systolic flow is 60-80 per cent of the diastolic flow during an equivalent time interval.

To substantiate that the area under the velocity curve can be used as an index of volume flow the actual flow has been measured (by a new type of constant pressure flow meter) within a few heart beats of the recording of the curves for establishing the velocity curve. Blood is infused at about the aortic diastolic pressure level. A sizable systolic as well as diastolic flow is found. When the aortic blood pressure is decreased from a normal to a hypodynamic level, the decrease in coronary flow as measured in cubic centimeters is of the same order of magnitude and has the same phasic relations as does that determined from the differential curves.

*The development of temperature control in infant rats.* ADDISON GULICK. Department of Biochemistry, University of Missouri, Columbia.

In infant rats the relation of metabolism to temperature differs sharply from what is found either in homeothermous or poikilothermous adult animals. For example, a rat six days old at rest in a glass chamber jacketed by a water bath at any temperature between 24°C. and 37°, will quickly acquire a rectal temperature approximately 1.3° to 1.8° higher than the air of the chamber. If chilled for an hour or more to 20° or lower, the rectal temperature will drop to less than 0.5° above the air of the chamber. During a 15° fall of body temperature the gas exchange drops to as low as 25 per cent of the initial normal (viz.,  $Q_{10}$  = roughly abt. 2.5). This seemingly poikilothermous picture is sharply refuted by a basal metabolism (warm chamber) ranging from 20 to 30 Cal. per sq. m. per hour during the first 8 days of life. This is about 9 to 14 Cal. per kgm. Undernourished rats may drop as low as 7 Cal. per kgm.

Between the ages of 9 and 12 days the rats begin to show muscular reflexes related to temperature, moderate coolness being a stimulus to active movements that raise the body temperature, although chamber temperatures of 20° and downward render the rats torpid.

From about 12 days this incipient temperature control increases rapidly. At 16 days the rats have a considerable degree of temperature regulation while muscularly active, but only slight beginnings of the ability to overcome chilling when not muscularly active. After this date they differ from the adult in the degree rather than the nature of their metabolic control. A 25 day rat in a 5.5° chamber can hold its temperature up to 34° for considerable periods by bunching into a ball and raising its metabolism to above 90 Cal. per square meter (Lee's formula). Adult normal is not strictly thermostable, but approximates 36° to 37° in surroundings in which the rats seem comfortable.

Charts are presented illustrating the responses at different ages.

*The effect of barbital derivatives on the electrocardiogram.*<sup>1</sup> ROBERTA HAFKESBRING and WINONA MACCALMONT (by invitation). Department of

<sup>1</sup> Aided by a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

Physiology, Woman's Medical College of Pennsylvania, Philadelphia.  
(Read by title.)

The effects of anaesthetic doses of several barbitol derivatives on the electrocardiograms of cats and dogs were studied. One hundred seventy-five experiments have been completed to date. Of these, 70 were controls, 54 with Nembutal, 29 with sodium amytal, and 22 with sodium barbital (repeated experiments on individual animals at seven day intervals).

The animals were trained so that normal electrocardiograms could be obtained. They stood in trays of warm salt solution. In each tray was a small porous cup holding a coiled silver wire electrode connected to a lead wire from electrocardiograph. During anaesthesia, the animal was supported in a hammock. Normal tracings were taken and the drug injected intraperitoneally. When fully relaxed, a second tracing was made and others at fifteen minute intervals (Nembutal) and one hour intervals (Barbital) until recovery.

Normal dog tracings showed several outstanding characteristics. Most showed marked sinus arrhythmia. There was "T" wave inversion, particularly in leads II and III, in the large majority of cases. ST segments were seldom isoelectric.

Nembutal. Six dogs and one cat used for Nembutal studies showed a marked increase in heart rate and a disappearance of the sinus arrhythmia. This seemed to be correlated with the original amount of vagus tone. PR intervals showed a definite decrease, but this can be accounted for by the increase in heart rate. QRS intervals were not prolonged, and there was no apparent slurring or notching of R waves. In many cases, T waves were changed in direction compared with normal, but whether great significance should be attached to this fact is doubtful because so little is known of the normal variation of T wave direction in dogs and cats.

Sodium Barbital. (Four dogs and three cats.) There was constant change in rate, even in the same animal. T wave tends to reverse in direction.

Sodium Amytal. (Four dogs and two cats.) All showed an increase in rate and regularity with the anesthetic. There was some evidence of T wave inversion. Both barbital and amytal experiments are still in progress.

No outstanding irregularities in rhythm or conduction were noted with any of the drugs used.

*Muscular efficiency as affected by taking breakfast and by the height of the respiratory quotient immediately before exercise.* JOHN HALDI, GEORGE BACHMANN, C. ENSOR (by invitation) and W. WYNN (by invitation). Laboratory of Physiology, Emory University School of Medicine, Atlanta, Ga.

Haggard and Greenberg recently reported a much higher muscular efficiency after breakfast than in the fasting state. They believe that there is a direct relationship between muscular efficiency and the level of the respiratory quotient before exercise. In studying the metabolism of glucose and of fructose we have observed that these sugars taken immediately or thirty minutes before exercise had no appreciable effect on muscular efficiency. In view of these apparently contradictory results we have conducted several experiments under conditions comparable to those of the experiments of the above mentioned investigators.

Two male adults well trained in work of this nature served as subjects. Upon arriving at the laboratory at 7:00 a.m. the subject reclined for 15 minutes. He then sat at rest on the bicycle for ten minutes while his gaseous exchange was determined. This procedure was followed before each exercise period. Exercise was then performed for 15 minutes on a Prony brake bicycle ergometer, work being done at a constant rate of 550 kgm.m. per minute. During exercise the gaseous exchange was determined as before exercise by the open circuit method of Carpenter and Fox. An average breakfast was then taken. One hour later, and again at two more hourly intervals, the above procedure was repeated. Control experiments were run in exactly the same way without the subject taking breakfast.

As uniform results were obtained on the two subjects, the averages of the data on one only will be given. The net muscular efficiency before breakfast was 24.2 per cent, and one, two, and three hours after breakfast 23.0, 24.1, and 23.5 per cent respectively. The respiratory quotients immediately preceding exercise were 0.78, 0.83, 0.78 and 0.78, respectively. In the control experiments with no breakfast the net efficiency during exercise which was taken at the same time as in the breakfast experiments was 23.9, 24.1, 24.1, and 23.8 per cent, respectively, and the respiratory quotients before exercise 0.77, 0.77, 0.74, and 0.74, respectively. Muscular efficiency therefore was not increased by taking breakfast nor could it be correlated with the respiratory quotient obtained immediately before exercise.

*Bio-assay of male hormone by gross weight of seminal vesicles and prostate gland from immature castrate rats.* S. R. HALL (introduced by E. I. Evans). Bureau of Dairy Industry, Division of Nutrition and Physiology, United States Department of Agriculture, Washington, D. C.

The use of rats in this assay has given widely different results in several laboratories. Some workers have claimed that a method based on weight increase of accessories was not reliable.

The author used male rats of the same stock (not necessarily litter mates) castrated at 24 days of age. Injections were started not less than 28 days later. After seven daily injections, the accessory organs were fixed, washed and weighed.

By this method synthetic testosterone (Ciba) in olive oil was assayed. In this particular stock of rats, a daily dose per rat of five gamma testosterone in  $\frac{1}{4}$  cc. olive oil gave an average response in five rats of approximately 100 per cent increase over castrate controls for both the ventral lobe of the prostate and the seminal vesicles. Doses from 5 to 320 gamma gave similar curves for each of these accessories. The seminal vesicle dose-response curve, however, is smoother and is considered satisfactory for the assay of testosterone.

The augmenting or so called X factor or factors present in this pure sample of olive oil was measured and plotted. Increasing the volume of the oil from  $\frac{1}{4}$  to  $\frac{1}{2}$  cc. daily at the level of forty gamma testosterone gave a greater response than doubling the dose of the hormone. The oil alone was ineffective. Similar but not identical results were obtained with other samples of olive oil.

The ratio between the ventral lobe of the prostate and the seminal vesicles maintained an approximate one to one ratio at all dose levels.

This relationship has been shown by others to be an important biological index.

*Production of testicular descent in monkeys by male hormone substance.*

JAMES B. HAMILTON (introduced by H. E. Himwich). Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, N. Y. (Read by title.)

In 9 immature male macaques, in which cryptorchidism exists until puberty, descent of the testes was obtained by administration of male hormone substances, testosterone acetate and testosterone propionate.<sup>1</sup> Descent was obtained after 14 days and apparently was due to processes of 1, growth and elongation of spermatic cord and vessels, and of cremaster muscle, sufficient to permit the testis to reach the scrotum; 2, development of the scrotum to receive and retain the testes.

Clinical application of male hormone in therapy of cryptorchidism may prove of value in 1, effecting testicular descent; 2, facilitating and simplifying surgery in cases where descent is prevented by mechanical or other factors, since the two chief difficulties in orchiopexy, namely, shortness of inguinal structures and a rudimentary scrotum, are overcome by growth of these structures following male hormone administration; 3, cryptorchidism of early childhood where an early and accentuated "pubertal state" may be induced to a, cause descent in those testes where mechanical factors do not interfere, and to b, designate cases where puberty may not be expected to result in spontaneous descent. Thus by inducing descent or by indicating the necessity for surgery the cryptorchid state may be treated early in life, thereby minimizing the incidence of hernia, testicular atrophy, pain and other conditions attendant upon cryptorchidism. Testicular descent obtained with anterior pituitary lobe extracts is probably due to stimulation of male hormone production, which is the agent actually responsible for the descent. Advantages of male hormone substance over anterior pituitary lobe substance are its pure, synthetic nature, stability, less widespread endocrine effects throughout body, non-dependence upon presence of functional testes. No carcinogenic or other untoward action has been observed in the experiments done thus far.

*Some applications of a differential manometer.* W. F. HAMILTON and R. A. WOODBURY. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

The "hypodermic manometer," described previously, registers pressure through the bulging of a plate and the movement of a mirror. If the cannula of this manometer is placed in a pulmonary vein it registers pressure fluctuations which reflect the action of the heart and the action of the respiratory muscles, transmitted by changes in intrathoracic pressure. In the hope of eliminating these latter fluctuations a differential manometer was so constructed that it registered the difference in pressure between the thoracic and the pulmonary venous pressures, i.e., the pulmonary venous pressure minus the intrathoracic pressure.

The device consists of an air tight chamber, fronted with an optical glass window screwed on to the front of the usual manometer. This chamber is connected by an air tube to a lax balloon in the chest while the

<sup>1</sup> Furnished by Ciba Company under the trade name Perandren.

usual cannula is connected with the pulmonary vein. The system then is a circular one with the wall of the vein separating the venous and intrathoracic pressures and the silver plate being subject to and registering the same identical stress. This shall be referred to as the net pulmonary venous pressure, the pressure which distends the wall and fills the heart.

Records will be presented which show that there is little or no variation in net pulmonary venous pressure though the gross pulmonary venous pressure may vary considerably. Certain fluctuations occur in the net pulmonary arterial pressure whose significance will be discussed as well as the respiratory fluctuations in the net arterial and venous pressures on the systemic side.

A second type of apparatus has been designed where the pressure is transmitted by fluid to both sides of the silver plate. This necessitates filling the anterior chambers with fluid and transmitting pressures to it with the usual leaden tube. The fluid in the anterior chamber introduces an asymmetrical refractive medium into the optical system, whose effect must be counteracted by trial lenses and prisms in order to get a photographable image.

Preliminary recordings will be presented, including a simultaneous record of blood pressure, intraocular pressure, and the net pressure in the intraocular arteries.

*A differential manometer and methods of measuring blood pressure curves.*

W. F. HAMILTON, R. A. WOODBURY and PHILIP DOW (by invitation).  
Department of Physiology and Pharmacology, University of Georgia  
School of Medicine, Augusta. (Demonstration.)

A differential manometer will be demonstrated together with some records and a method of measuring them.

*Degeneration of the supra-optic nucleus following hypophysectomy in the dog.*

KENDRICK HARE (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City.

Serial sections were made of the diencephalon of each of 10 dogs which lived from 25 to 233 days after removal of the pituitary. Microscopic examination revealed pronounced degeneration in the supra-optic nuclei in all cases. Although more than half its cells had disappeared, the rostral portion of the nucleus suffered least. The caudal parts of the nucleus had wholly disappeared or consisted of very few cells. The death of these neurons was not the result of infarcts involving the chiasmal region, nor is it likely that direct trauma to the nerve cell bodies was responsible, for neurons much nearer the lesion were spared. It is believed that their destruction was brought about by the removal of their processes passing into the pars nervosa of the pituitary.

*Specific reflex afferents in mammalian plantar nerves.* A. SIDNEY HARRIS (introduced by A. D. Keller). Department of Physiology and Pharmacology, University of Alabama.

Faradic stimulation of low threshold cutaneous fibers in the medial plantar nerve or the superficial branch of the lateral plantar of the reflex dog gives rise to a response pattern of ipsilateral extension consisting of contraction of extensor muscles and the plantar flexors of the limb. The response has occurred in all animals studied without exception. This

series includes: 1, animals with brain stem transections in which the plane of transection entered dorsally between the anterior and posterior colliculi and emerged ventrally at levels ranging from the posterior margin of the anterior fourth of the pons forward to the mammillary bodies; 2, low spinal animals; 3, animals without central nerve axis operation but under the influence of nembutal. The quality of the responses was independent of neural bias. Some of the decerebrate animals showed a high degree of extensor rigidity and others were almost atonic, depending upon the level of the operation.

The decerebrate, spinal, and nembutalized animals responded qualitatively alike. Attempts to elicit ipsilateral extension responses from the main trunk of the lateral plantar nerve have been unsuccessful. The nerves yielding the ipsilateral extensor response are distributed almost entirely to the skin of the planta including the pads and the skin between them. Reflexes evoked by the medial plantar have been subjected to the more thorough study. Repeated thyatron shocks within a wide range of frequencies applied to it evoke the ipsilateral extension response at low threshold. More intense shocks superimpose a response of flexor muscles upon the extension pattern but there is not a true reversal. Through a distinctive low threshold zone the extensor pattern appears to be uncontaminated by any non allied activity. The action potentials of the fibers making up the "pure sample" of ipsilateral extensor afferents in the medial plantar nerve can be recognized on the cathode ray oscillograph as a small elevation composed of very few fiber potentials. At about the threshold of the flexor response a widening occurs. With increased intensity of stimuli this second component grows and becomes an elevation much higher than the faster process.

In histological preparations the few larger fibers stand out strikingly.

*Quick and delayed reflex responses evoked from depressed mammalian nerve.*

A. SIDNEY HARRIS (introduced by A. D. Keller). Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

Experiments with aseptically decerebrated dogs were continued over periods up to five days. A stretch of tibial nerve 6 or 7 cm. long was dissected free and sectioned at the distal end. It was kept moist with Locke's solution and the whole wound sterilized with chlorazene. During the first day reflex responses evoked by stimulating the nerve were of the classical flexion reflex type, but during the second or third day modifications occurred causing upon effective stimulation unusual reactions. Irritability was depressed in the distal part of the free nerve so that no response to thyatron shocks of 0.2 ms. duration appeared. Galvanic stimuli evoked reflex responses with a latency of 600 to 800 ms. in which flexors and extensors of the same limb ordinarily contracted simultaneously. Galvanic stimuli applied to a more proximal, less depressed part of the nerve evoked both the quick flexion reflex with extensor inhibition and the delayed response described above. When repeated thyatron shocks evoked any response from this area it was the quick reflex only. Responses from more proximal parts of the nerve were as from freshly dissected nerve.

The nerve fibers which evoke the delayed reflex which appears "diffuse" or incoordinate have not been identified oscillographically, but it appears probable that the depression is of the type caused by asphyxia in which

the large fibers fail first and the small ones last (Clark, Hughes and Gasser, *Am. J. Physiol.* **114**: 69, 1935). This suggests that it is the smallest or C afferent group that evokes the delayed response. The ineffectiveness of brief thyatron shocks as compared with galvanic stimuli, and the high threshold also seem to support this idea. It is further suggested that in the partially depressed nerve yielding the quick and delayed reactions some of the fibers in the B range remain functional along with the C group, all suffering some diminution in reactivity. Upon stimulation of normal nerve some influence from larger fibers perhaps inhibits the delayed response.

*The discharge of impulses in the optic nerve fibers of the eye of Pecten irradians.*

H. K. HARTLINE. Johnson Foundation, University of Pennsylvania, Philadelphia.

The discharge of impulses in the optic nerve fibers of the eye of the scallop, *Pecten irradians*, has been studied by recording their amplified action potentials. In the whole optic nerve the response to illuminating the eye is strongest at the onset of illumination, diminishes distinctly after several seconds, but nevertheless continues as long as the light shines. When the light is turned off there is another strong outburst of nerve impulses, lasting several seconds.

The *Pecten* retina contains two distinct layers of sensory cells each giving rise to a separate branch of the optic nerve. It seems probable that both the proximal and distal layers consist only of primary sensory cells and interstitial cells, that there are no other nerve cells in the eye, and that the axons of the sensory cells pass directly into the optic nerve without the intervention of any synapse (Schoepfle and Young, *Biol. Bull.* **71**, 403).

By recording from the appropriate branch of the optic nerve the responses of the two different sensory layers may be studied separately.

Impulses are discharged in the fibers from the proximal sensory cells only when the eye is illuminated. They cease when the light is turned off. Records from single fibers show a regular series of impulses beginning at a high frequency and adapting rapidly to a level which is maintained as long as the light shines. The frequency is higher the greater the intensity of illumination.

Impulses are discharged in the fibers from the distal sensory cells only in response to cessation of illumination. This discharge may last many seconds; it is abruptly stopped if the eye be re-illuminated. Records from single fibers show that the frequency of the discharge is initially high, but diminishes rapidly. The level of frequency and the duration of the discharge are greater the more intense and more prolonged the preceding illumination. In some cases, following strong illumination, the discharge in these fibers may break up into rhythmic bursts of impulses, the bursts occurring synchronously in all the fibers of this branch of the optic nerve.

*The effect of adrenal cortical extract on renal excretion.* F. A. HARTMAN,

LENA LEWIS (by invitation) and GWENDOLINE TOBY (by invitation).  
Department of Physiology, Ohio State University, Columbus.

It has been shown by Thorn, Garbutt, Hitchcock and Hartman that intravenous injection of large amounts of cortical extract produces a differential effect on the excretion of electrolytes in the normal human being. This work has been continued with human beings and dogs as

subjects. A constant diet has been maintained during the experiments. The dose for the threshold effect varies considerably in different individuals. In the human being the effect of an injection persists for 6 to 8 hours, while in dogs it seems to persist for a longer period. In the human being the maximum is reached in 3 to 5 hours. Successive intravenous injections of the same dose seems to be less effective in the dog.

*Osmotic and surface properties of bacteria.* E. NEWTON HARVEY and FRANK H. JOHNSON (by invitation). Princeton University, Princeton, N. J.

When luminous bacteria, which normally live in sea water, are placed in distilled water the luminescence and motility cease, the suspension becomes permanently foamy and clearer and the bacteria are difficult to centrifuge. Cytolysis (bacteriolysis) appears to take place. The process differs from that of most cells in that not even momentary (0.1 sec.) swelling occurs. Centrifuging at 100,000 to 250,000  $\times$  gravity shows that the total volume of cells in various sea water dilutions is always less than in sea water, becoming some 20 per cent less in 1 per cent sea water. The total number of bacteria by dark field counts is approximately the same in 1 per cent and undiluted sea water, showing that no bacteria dissolve completely. Densimeter experiments (photronic cell measurements of light transmitted) show progressively more light transmitted in diluted sea water suspensions, reaching a maximum in distilled water. If traces of Ca or Mg are added, less light is transmitted, the suspension looks more turbid and the bacteria centrifuge well. The effect is reversible. Dark field observation shows that the Ca and Mg affect the surface of the bacteria which appear to be surrounded by a salt sensitive colloid layer (capsule?) whose thickness, surface texture and refractive index determine the total volume, rate of sedimentation and optical appearance, respectively, of the suspension in various sea water dilutions and salt solutions. Ca and Mg cannot prevent loss of luminescence and motility or foaminess, which are due to loss of substances from the bacterium.

The wall of the bacterium appears to be rigid but centrifuge experiments show that the total volume of bacteria decreases in concentrated sea water to a maximum of 27 per cent in 2.5 times sea water, beyond which no more water can be withdrawn.

*Ovulation in the rabbit following upon stimulation of the hypothalamus.*

H. O. HATERIUS and A. J. DERBYSHIRE, JR. (by invitation). Physiological Laboratory, Ohio State University, Columbus.

Ovulation has been induced by electrical stimulation of a definite area in the hypothalamus. Bipolar electrodes were employed, insulated to the small blunt tips, with an inter-electrode space of less than 0.5 mm., the exposed area of each electrode being not more than 0.25 sq. mm. Two dry cells supplied the current. Stimulation at a depth of approximately 5 mm. below the surface of the thalamus evoked a slowly developing flexion of the hind limbs and pelvis, accompanied by shortening of the trunk and, frequently, elevation of the tail. An inductorium coil distance of 9 cm. usually determined the minimum strength required to elicit the motor pattern; however, the stimulus was continued arbitrarily for one-half hour at 7 to 8 cm. coil distance. Despite the increased strength of stimulation, the motor activity was confined to an area 2 or 3 mm. in dorso-ventral extent, indicating the degree of stimulus "spread." The point

of stimulus involved in the ovulatory response has been localized by the motor response, although there is as yet no evidence of a relation between the two.

Histological studies in progress indicate that a circumscribed area directly above and anterior to the optic chiasma is involved. Examination of negative cases reveals the lesions a short distance away from this region; localization of electrodes apparently is a critical factor, since in some at least of the negative cases the lesions lie within  $1\frac{1}{2}$  mm. of those found in our positive experimental animals. Failure to ovulate in these instances, therefore, constituted an excellent control, since it would appear to preclude the factor of the manipulation involved and, moreover, would indicate a highly localized region related to the ovulatory mechanism.

*Visual intensity discrimination in different parts of the spectrum.* SELIG HECHT, JAMES C. PESKIN (by invitation) and MARJORIE PATT (by invitation). Laboratory of Biophysics, Columbia University, New York City.

We have measured our own visual intensity discrimination with white light and with different parts of the spectrum isolated with Wratten monochromatic filters. The eye is first adapted to a field of a given intensity  $I$ ,  $40^\circ$  in diameter. The intensity of a central  $12^\circ$  portion of the field is then increased for a fraction of a second, and the minimum intensity increment  $\Delta I$  is found which is just perceptible with certainty as an increase in brightness.

Contrary to the classically accepted measurements of Koenig and Brod-hun, we find with Aubert, Blanchard, and with Steinhardt that the relation of  $\Delta I/I$  to  $I$  over the whole visual range using white light shows two distinct steps, a low intensity portion and a high intensity portion.

The identification of these two sections with rod and cone activity respectively is confirmed by the behavior of the function with different parts of the spectrum. With red light the relation between  $\Delta I/I$  and  $I$  is continuous and single for the whole intensity range. With shorter wavelengths the low intensity section makes its appearance, and becomes larger as the light goes toward the blue. With extreme violet light, the low intensity section is almost the same size as the high intensity section. This behavior in the spectrum is entirely in keeping with the relative spectral sensibility of rods and cones, as separately determined, and conforms with the well known photochromatic interval.

The two separate relations in intensity discrimination may be described in terms of the familiar idea that the photoreceptor process is basically a cyclic pseudoreversible system composed of a photochemical and a dark reaction. The equations describing such a system fit the data of intensity discrimination obtained not only by us, but by Aubert, by Blanchard, by Steinhardt, and by Smith.

*Prolongation of pregnancy in the rabbit by injection of progesterone.* GEORGE P. HECKEL (by invitation) and WILLARD M. ALLEN. Departments of Obstetrics and Gynecology and of Anatomy, The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Parturition has previously been delayed in the rabbit by injection of crude corpus luteum extracts—preparations known to contain progesterin or presumed to contain it because of their method of preparation.

In our experiments both natural and synthetic progesterone were used. One milligram of the crystalline material is equivalent to one international unit. Dosage ranged from 0.25 mgm. to 2.0 mgm. per day, and injections were begun on the 20th, 25th or 28th, day of gestation and continued until parturition occurred or until it had been significantly delayed beyond the usual time for delivery (32 days).

Pregnancy was prolonged in 9 out of 10 animals receiving 1.5 mgm. or 2.0 mgm. per day. And in 4 out of 6 cases in which there was no operative interference the mechanism of parturition was upset, since the length of time between delivery of the first and last fetus, usually little more than an hour, was as long as three days. In nearly all cases in which parturition occurred after the 34th day the fetuses appeared postmature, judging from their size and amount of hair. Living fetuses were not obtained after the 35th day. In cases where injections were stopped before parturition had occurred, delivery began within 24 to 48 hours. Histological examination of the ovaries at the end of the experiments showed the corpora lutea to be atrophic, indicating that the prolongation of pregnancy was not due to survival of the animal's own corpora lutea.

*The fractionation, and a study of the interaction, of the gonadotropic factors in pregnant mares' blood.* ARTHUR A. HELLBAUM (introduced by Edward C. Mason). University of Oklahoma.

The ovary stimulation complex of pregnancy mare blood has been fractionated into three components, whose separate effects induce: 1, follicular development; 2, luteinization, and 3, augmentation. The follicular stimulating fraction produces only follicular development in the ovaries of both normal and hypophysectomized immature rats. However, when injected in combination with the luteinizers of either the pregnancy blood or pituitary, extensive luteinization always follows.

The concentration of the luteinizing principle in pregnancy mare blood is relatively low but its physiological action is similar to that of the pituitary luteinizing fraction. The third or augmenting fraction is a normal constituent of non-pregnant as well as pregnant mares' blood and has no activity by itself; however, it is capable of enhancing or augmenting the gonadotropic action of the pituitary complex. This augmenting principle, interacting with definite amounts of the follicular stimulating and luteinizing factors, invokes a response which is characteristic of the blood of mares during pregnancy.

*The relation of the center of gravity to the base of support in stance.* FRANCES A. HELLEBRANDT, GENEVIEVE BRAUN (by invitation) and RUBY H. TEPPER (by invitation). Department of Physiology, University of Wisconsin Medical School, Madison.

To facilitate the physiological study of the upright posture of man a method has been devised whereby the movements of the center of gravity in the vertical orientation planes may be graphically recorded and projected into the base of support as a function of time. Observations thus far made on 60 normal subjects of both sexes ranging in age from the first to the seventh decade indicate that sway is inseparable from the vertical stance. It varies in magnitude. The average shift of the center of gravity is confined to a relatively small fraction of the total area of underpropping. In the most unstable subjects it approaches the fore extremity of the

anterior diameter of structural foot support but never encroaches dangerously upon the limits of lateral or posterior support. The average location of the center of gravity during three minutes of quiet standing in a natural comfortable stance with the heels slightly separated and the toes turned out just enough to equalize the antero-posterior and transverse diameters of support is so placed as to be surrounded by a roughly symmetrical margin of static security. The intensive study of a small group of young adult women with an exceptionally developed kinesthesia indicates that the center of mass may be sustained in a remarkably constant average location in relation to the structural supports, irrespective of the magnitude of postural sway.

The maintenance of the upright stance is a dynamic phenomenon. Gravitational rotatory stresses must be equilibrated by postural tone. It is suggested that the incessant shift of the center of gravity may serve as a constantly varying stimulus flowing over the afferent limb of the geotropic reflex and that herein may reside the mechanism for the functional rotation of motor units conceded to be responsible for the relative indefatigability and economy of postural tone.

*A study of panting in normal unanesthetized dogs heated by diathermy.*  
ALLAN HEMINGWAY and H. G. BARBOUR. Yale University, New Haven, Conn.

By using high frequency diathermy current it is possible to give to an animal any desired amount of heat at a controlled rate of heating. Using measured heat dosages panting has been induced in normal unanesthetized dogs under ordinary laboratory conditions. Two types of measurement have been made. In type I, skin and body temperatures have been measured at various positions with Lewis type thermocouples and panting has been studied by chest movement which gives rate quantitatively and relative depth of respiration qualitatively. In the type II, an improved type of plethysmograph has been used to measure ventilation rate and tidal air volumes. This method gives an accurate measure of ventilation. This method has the advantage that the animal breathes in a normal manner eliminating the use of a mask or a tracheal cannula.

When induced by diathermy panting commences after a definite caloric dose which seems to be independent of the rate of heating or the absolute body temperature but does depend on environmental conditions.

When panting commences there is increased total ventilation, a very marked increase of rate from the normal basal values of 10 to 20 per minute to values of 200 to 300 per minute. There is decreased tidal volume associated with the rapid rate. During incipient panting periods of the normal type of respiration may alternate with panting. In other cases periods of apnea alternate with periods of panting.

The results support the theory that the heat stimulation causes a rapid air movement over the moist surfaces of mouth, tongue and pharynx without too great an air movement in the alveoli of the lungs.

*Hemoglobin and the alkali of the blood.* YANDELL HENDERSON and LEON A. GREENBERG. Laboratory of Applied Physiology, Yale University New Haven, Conn. (Read by title.)

Our questions were: 1. How much additional base can the hemoglobin of the blood combine with? In other words, how much  $\text{CO}_2$  can it liberate

by Pflüger's reaction? 2. How much base is it already combined with? Van Slyke and his co-workers have made such determinations chiefly upon crystallized hemoglobin and within the physiological range of pH. (J. Biol. Chem. **54**: 481, 507, 1922; **60**: 89, 1924.)

We used dog's blood of an oxygen capacity of 19 volumes per cent, and 40 volumes per cent CO<sub>2</sub> at 40 mm. Hg. NaHCO<sub>3</sub> in solution was added, and the blood was evacuated in a Van Slyke apparatus until no more CO<sub>2</sub> could be liberated. The remaining amount of BHCO<sub>3</sub> was then determined by the addition of acid and evacuation. The CO<sub>2</sub> originally in the blood as BHCO<sub>3</sub>, plus the amount of CO<sub>2</sub> added as NaHCO<sub>3</sub>, minus the amount of CO<sub>2</sub> obtained after the addition of acid indicated that the maximum that could be liberated by hemoglobin was 150 volumes per cent of CO<sub>2</sub>.

This figure corresponds to  $\frac{150}{19} = 7.9$  atoms of alkali for every molecule of oxygen that hemoglobin can hold.

In order to estimate the total amount of base held by hemoglobin, 5 cc. of blood were placed in a cellophane sack, held to a flat shape only 2 mm. thick, but with a surface of 50 sq. cm., and were dialyzed for 72 hours in 150 cc. of water or saline previously supersaturated with CO<sub>2</sub> in a "spark-let" bottle, and kept tightly stoppered in the refrigerator. The dialysate was titrated to determine the amount of alkali obtained from the blood. It corresponded to 200 volumes per cent of CO<sub>2</sub>—40 from the BHCO<sub>3</sub> and 160 from the BHb—indicating  $\frac{160}{19} = 8.4$  atoms of alkali in BHb per molecule of oxygen capacity: rather less than was estimated by Haggard and Henderson (J. Biol. Chem. **45**: 199, 1920).

These figures indicate 1, that the capacity of hemoglobin for alkali is about 16 times its capacity for oxygen; 2, that in blood hemoglobin is a little more than 50 per cent saturated with alkali; and 3, that about 80 per cent of the alkali of the blood is held by hemoglobin.

*Experimental analysis of the thermostromuhr for small flows.* J. F. HERRICK, E. J. BALDES and F. P. SEDGWICK (by invitation). The Mayo Foundation, Rochester, Minn.

If one wishes to use Rein's Thermostromuhr quantitatively he must appreciate the limitations, especially in regard to the following:

- 1, the time necessary for the establishment of thermal equilibrium, and
- 2, the deviation from the equation  $G \cdot V^\beta = K$  when measuring small flows.

The time required for the establishment of thermal equilibrium between thermojunctions, blood vessel and blood is about a minute. Attempts to interpret changes in flow which occur in less time may not be reliable.

It has been shown that the above equation of a hyperbola represents the relation between V, the blood flow, and G, the deflection of the galvanometer in the thermocouple circuit. From the equation it is obvious that when the flow is zero the deflection of the galvanometer is infinite. However, from a detailed consideration of the principle underlying the method it is shown that the deflection is zero when the flow is zero. This apparent discrepancy for zero flow can be accounted for if one assumes that the actually observed points start to deviate from the theoretical hyperbola after a certain minimal flow, and lie on a curve which reflects back through

the origin. The experimental curve showing this deviation which takes place between the flows of 3 cc. per minute and zero is given. The curve is similar to that theoretically determined by Burton for the Rein Thermstromuhr and that experimentally obtained by Jongbloed and Noyons with their Aerothermorheograph.

Since the deflection of the galvanometer approaches zero for very small flows and becomes zero for cessation of flow, whereas it usually decreases for increase of flow, one must be careful to distinguish between a cessation of flow and an increase. No difficulty will arise if sufficient time is allowed for the establishment of thermal equilibrium. One should avoid any interpretation of changes occurring in less than this required time. Certain controversies have arisen because this particular limitation was disregarded.

*The influence of diet upon urea clearance.* R. C. HERRIN, R. JOHNSON (by invitation) and K. SIEBECKER (by invitation). Department of Physiology, University of Wisconsin Medical School, Madison.

In adult dogs fed starch and Crisco, the ingestion of the non-saponifiable matter (N.S.M.) of 75 grams of butter with the test meal resulted in a 27 per cent increase in the average maximum urea clearance. In 3 dogs, receiving an ordinary biscuit diet, the effect of the N.S.M. of 75 grams of cod liver oil or fat 3 rendered from meat residue was tested for its effect on urea clearance about 20 hours after the last biscuit feeding. Fat 3 increased the clearances in all dogs, the percentage increase ranging from 13 to 100. Cod liver oil in one dog resulted in increases of 11 to 84 per cent and in another whose clearance was not affected within 4 to 6 hours there was a 100 per cent increase 9 hours post-prandially.

Three other dogs were placed on this diet: tankage 100, starch 21, Wesson oil 5, yeast 4, NaCl 1, CaCO<sub>3</sub> 0.5. The tankage, a protein concentrate, was extracted with ethyl ether and the yeast with anhydrous ether. The dogs were exposed to ultra-violet light periodically and received crystalline carotene equivalent to 7000 units of vitamin A daily. The average daily protein intake exceeded 200 grams. The maximum urea clearances of plasma, per square meter of body surface, 20 hours after the last feeding were as follows. Dog 1, biscuit diet—44 cc.; after 22 days on the experimental diet—34; after 50 days—17; after 63 days—31. Dog 2, Crisco-starch diet—25 cc.; after 37 days on experimental diet—28; after 52 days—35. Dog 3, Crisco-starch diet—47 cc.; after 37 days on experimental diet—46; after 52 days—47. These results are in marked contrast to those reported by other workers for fresh meat feeding.

*Observations on the finger volume pulse recorded photo-electrically.* ALRICK B. HERTZMAN and CLAIR R. SPEALMAN (by invitation). Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

Variations in the light transmission of a finger due to changes in the blood content may be detected by a photo-electric cell and recorded optically by means of an amplifier and string galvanometer. The arrangement permits recording either of details of the finger volume pulse (F.V.P.) curves or of slower developing changes in finger blood volume (F.B.V.) due to constriction, etc. Although amplification and string protection affect the sensitivity and the recorded height of the F.V.P., the essential form of the curve remains unchanged. The method appears to be applicable to other areas which may be transilluminated.

The F.V.P., thus recorded, shows greater detail of form than do optically recording finger plethysmographs. It resembles the optically recorded radial pulse. It is essentially triangular in form, the anacrotic limb rising steeply, the catacrotic limb falling more gradually. A well defined dirotic wave appears about one-third of the distance down on the catacrotic limb. The form of the F.V.P. curve appears to be characteristic of the individual.

The form of the F.V.P., the position of the dirotic wave and the degree of dirotism change with circulatory conditions. Thus, in a case of essential hypertension, the F.V.P. curve was rather rounded, the rise gradual, and the dirotic wave absent.

Ordinary quiet breathing affects only slightly the F.V.P. and the F.B.V. Vasomotor waves in F.B.V. are often present without an important effect on the F.V.P. Local cold diminished the F.V.P. without significant effect on the form of the curve; heat has the opposite effect.

Voluntary apnea decreased both F.B.V. and F.V.P. Expiration against a closed glottis increased F.B.V. and decreased F.V.P. Inspiration against closed glottis decreased both F.B.V. and F.V.P.

The response to exercise of the legs was complex: marked decreased in F.V.P. immediately after cessation of exercise followed by a marked augmentation resulting in a maximum F.V.P. about one minute after the end of exercise, followed by a gradual decrease in the F.V.P. to the resting value.

As an example of drug action: Amyl nitrite decreased F.B.V. but markedly increased F.V.P.

*Relation of threshold of excitability of nerve to carbon dioxide tension.* J. P. HETTWER (introduced by J. A. E. Eyster). Department of Physiology, University of Wisconsin Medical School, Madison.

The effect of carbon dioxide at various tensions approximately within physiological limits on the minimal and maximal action potential threshold of frog sciatic nerve is graphically explored. Apparatus employed includes an amplifier feeding to a string galvanometer and condensers for stimulation. Results show a progressively increasing rise of both thresholds with increasing tensions of carbon dioxide applied either to the whole nerve or around the stimulating electrodes only. There is no indication of an optimum tension for threshold. When the gas is carefully applied around the leading-off electrodes only, there is no effect on threshold as determined outside and the magnitude of response is not significantly altered. The reported optimum for magnitude of response to supramaximal stimulation is not found.

*The effect of hypoglycemia on the metabolism of the brain.* H. E. HIMWICH and J. F. FAZEKAS (by invitation). Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

We have previously shown that the brain oxidizes carbohydrate exclusively as demonstrated by a R.Q. of unity and substantiated by absorption of glucose and lactic acid from the cerebral blood. Since the brain depends largely upon the blood for its supply of carbohydrate, it becomes important to determine the effect of hypoglycemia on the metabolism of the brain. Such studies gain added significance because of the new insulin therapy of schizophrenia. Fifteen dogs were anesthetized with amytal and blood samples drawn practically simultaneously from the longitudinal sinus and the femoral artery were analyzed for glucose and oxygen before and after the development of hypoglycemia. Before the injection of insulin the

average values of 13 observations for the utilization of glucose and oxygen were 8 mgm. per cent and 4.6 vol. per cent respectively. During the terminal hypoglycemia, characterized by parasympathetic overactivity, the average utilization in 10 observations was reduced to 3.1 mgm. per cent of glucose and to 4.01 vol. per cent of oxygen. It is probable that the reduced absorption of glucose and oxygen per 100 cc. of blood occurred despite a slower blood flow. Thus a correlation exists between the diminished absorption of glucose and a smaller oxygen consumption by the brain. The examination of muscle, however, which does not oxidize carbohydrate exclusively, revealed a diminished glucose absorption with no change in the oxygen consumption during hypoglycemia. Before insulin was injected the average values of 10 observations for the utilization of glucose and oxygen were 7.6 mgm. per cent and 6.91 vol. per cent respectively. In 13 observations during hypoglycemia the utilization of glucose was reduced to 1.5 mgm. per cent; nevertheless, the oxygen consumption remained practically the same, 6.04 vol. per cent. Further experimentation is now in progress on the changes occurring during the development of hypoglycemia.

*Studies on diaphragmatic sensation.* J. C. HINSEY and R. A. PHILLIPS (by invitation). Department of Physiology, Cornell University Medical College, New York City.

Cats were observed following recovery from etherization after these procedures: 1, section of the vago-sympathetic trunks in the neck and transection of the spinal cord at C.7; 2, removal of the right and left sympathetic chains from above the stellate to T.7 and transection of spinal cord at T.4; 3, removal of the right and left sympathetic chains from above the stellate through T.6, section of the right and left vagus in the chest, and transection of spinal cord at T.3 (cat observed next day); 4, removal of right and left sympathetic chains from above superior cervical ganglia to below T.8 and, three weeks later, transection of the spinal cord at T.4. In all instances, when appropriate stimulation was applied to the peritoneal surface of the diaphragm, evidence of nociceptive sensation was present. Stimulation of the margin (about 0.5 cm.) of the diaphragm resulted in ipsilateral diaphragmatic tetanus but no evident sensation. When both vago-sympathetic trunks were sectioned in the neck, spinal cord transected at T.7, and both phrenics sectioned just above the diaphragm (followed by artificial respiration), central stimulation of the individual phrenic nerves elicited nociceptive responses. It is concluded that the thoracolumbar and vagal pathways are not essential for nociceptive sensation from the central portion of the diaphragm.

Oemic acid and silver preparations of the phrenic nerve in which the sympathetic supply has been degenerated show a number of small myelinated fibers and fascicles of fibers that are either unmyelinated or very small myelinated ones. Degeneration procedures are in progress which will isolate the sensory component of the phrenic nerve.

*The effect of adrenal cortical extract on the respiratory metabolism of normal human beings under various conditions.* FRED A. HITCHCOCK and R. C. GRUBBS (by invitation). Department of Physiology, Ohio State University, Columbus.

The respiratory exchanges of 11 normal human beings (8 males and 3

females) have been determined by means of the Tissot-Haldane technic while the subjects were 1, in a basal condition; 2, standing erect, and 3, walking on a treadmill at a uniform rate. Experiments were carried out before, during and after the administration of relatively large amounts of adrenal cortical extract. The results indicate that adrenal cortical extract in sufficiently large doses tends to reduce the oxygen consumption of normal human beings. The degree of reduction is greater under conditions which increase the oxygen consumption. The effect on the basal oxygen consumption was so slight as to be without significance. Six of eleven subjects showed a lowered basal rate after the administration of adrenal cortical extract, the average for the group being only one per cent lower in the cortical experiments than in the control tests. On standing erect the oxygen consumption of the various subjects was increased from 10 to 30 per cent. Eight out of eleven subjects consumed less oxygen in standing erect after receiving adrenal cortical extract than in control tests. The average difference for the group was nearly 6 per cent. The rate of walking on the treadmill was approximately 100 meters per minute, which caused the oxygen consumption of the subjects to rise to a value from 3 to 4 times as great as that observed while they were standing still. Under these conditions the administration of adrenal cortical extract was followed by a reduced oxygen consumption in 9 of the 11 subjects. The average decrease for the entire group was about 14 per cent. These results seem to justify the conclusion that the administration of adrenal cortical extract to normal human beings tends to increase the muscular efficiency.

*The effect of staphylococcus aureus toxin on the heart.* H. E. HOFF, J. DINGLE (by invitation) and L. H. NAHUM. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

The exotoxin of staphylococcus aureus, titrated for its dermonecrotic and haemolytic activity was injected intravenously in eight normal and three anesthetized (sodium amytal) rabbits, in doses varying from 0.5 to 0.03 cc. per kilo. Doses less than 0.062 cc. per kilo produced no appreciable changes; this or more led to death in from 1½ to 20 minutes. Frequent electrocardiograms were taken and in three animals under anaesthesia, blood pressure and electrocardiograms were simultaneously recorded. All animals were autopsied immediately after death. Post-mortem examination showed pulmonary and hepatic engorgement, together with well-filled right and left auricles, and ventricles. This indicates that peripheral vascular failure was not an important factor in the death of the animals.

Blood pressure declined progressively until at levels of 30 to 40 mm. Hg convulsions and death occurred. During the period of decline in blood pressure electrocardiographic changes characteristic of severe myocardial damage were noted. These were: 1, elevation of the S-T take-off; 2, changes in the amplitude, direction and contour of the T wave; 3, disappearance of the S-T interval, and 4, shortening of the duration of the ventricular complex. These resembled closely the changes found in monoiodoacetic acid poisoning.

Incidental variations in rate, and in A-V conduction occurred during the decline of blood pressure, but were not of sufficient magnitude to account for failure of the circulation. In most instances the heart con-

tinued to beat for several minutes after syncope, but in three experiments arrest occurred almost immediately, and in two of these ventricular fibrillation was observed as a terminal event.

Death from staphylococcus aureus toxin is attributed to poisoning of the myocardium and the resulting failure of contractility.

*Effects of castration and of male hormone administration upon the responses of the rat to certain barbiturates.* HARALD G. O. HOLCK, MUNIR A. KAÑAN (by invitation), LUCILLE M. MILLS (by invitation) and EDWIN L. SMITH (by invitation). Universities of Beirut (Syria), Chicago and Nebraska.

After castration of adult male rats hypnosis was lengthened to a variable degree and deaths were more frequent following the administration of four barbiturates to which a sex-difference was exhibited (pernoston, 5-beta-bromallyl-5 sec.-butyl barbiturate; evipal, 5-cyclo-hexenyl-1,5-dimethyl; amytal, 5-ethyl-5-isoamyl; pentobarbital, 5-ethyl-5-alpha-methylbutyl). However, the response in no case became as long as that of the normal female rats. In case of 5,5-diethyl barbiturate (barbital) there was no sex-difference and castration did not alter the adult male rat response. Three to four week rats did not exhibit any sex-difference to evipal or to pentobarbital. Castration of such baby males did not immediately alter the quickly shortening response to evipal, but typical castration effects appeared as they were nearing maturity. Adult female rats which were spayed continued to give responses to evipal similar to those of control females.

Preliminary daily administration of male hormone from human urine or of testosterone acetate in oil for periods up to 5 weeks to normal or spayed female rats shortened the depression significantly in case of evipal and pernoston, but did not make it as short as that of normal males. The effect of castration of male rats was partly counteracted when male hormone was given immediately before and after this operation; evipal was used in these tests.

*The enhancement of muscular contraction after tetanus.* R. G. HORTON, D. T. WILBER and S. A. GUTTMAN (introduced by H. S. Liddell). Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

Neurohumoral action furnishes an explanation for the following results obtained from an isolated nerve-muscle preparation. If a sciatic-gastrocnemius preparation of frog (or cat), which has been partially fatigued by single stimuli repeated 1/sec., is stimulated for a short period (5 sec.) at a higher rate (20/sec.), on return to stimulation at 1/sec. the contractions are much larger than before tetanising; i.e., they are "enhanced."

Since myoneural fatigue is assumed to occur before muscular fatigue, it seems probable that the recovery is concerned with myoneural junction. This is substantiated by the fact that curare abolishes the phenomenon with direct stimulation. The work of Boyd on curare on the cat and of Lubinska on magnesium salts also indicates that the myoneural junction is the locus of the phenomenon.

The increase in action potential of nerve after tetanic stimulation observed by Waller (1896) and others remains to be correlated with our findings.

Since the phenomenon appears equally well after sympathetic degeneration the Orbeli mechanism is not involved.

Possible mediators of this effect are acetylcholine (Dale and others) or an adrenaline-like substance (Corkill and Tiegs) from some other mechanism than the sympathetic nerves.

The phenomenon appears only within a certain range of tetanising voltages and loads. High voltages or too heavy loads cause a temporary suppression of contraction after tetanus with a subsequent recovery to normal or even enhanced contractions. Enhancement may also be produced by three stimuli of high voltage one second apart but more than three usually result in suppression of contraction.

*The effect of adrenalectomy on the metabolic response of the albino rat to cold.*

STEVEN M. HORVATH (introduced by F. A. Hitchcock). Department of Physiology, Ohio State University, Columbus.

Experiments have been carried out on the albino rat to determine the probable rôle of the adrenal gland in the increased heat production resulting from exposure to cold.

Sixteen young male animals were kept at an environmental temperature of 27-28°C. and their basal heat production measured at 29°C. They were then placed in the cold (4°C.) for periods ranging from 1 to 72 hours, and their metabolism under basal conditions measured again at 29°C. This procedure was repeated following both unilateral and bilateral adrenalectomy.

A second series of experiments was conducted, subjecting the rats to the same procedure outlined above except that the heat production was measured at 4°C. instead of at 29°C.

No measurable difference was found in the basal heat production at 29°C. of normal animals and those having only one adrenal. In animals with both adrenals removed there was a lowered basal metabolism (average 4.5 per cent).

Normal animals showed a 24.8 per cent increase in their metabolism following exposure to a temperature of 4°C. After the removal of a single adrenal, cold resulted in a stimulus averaging 14.0 per cent. Double adrenalectomized animals failed to show any stimulation above their basal metabolic rates following exposure (1 to 1½ hours).

The metabolism of normal rats at a temperature of 4°C. rose 180 per cent or higher above basal rates measured at 29°C. After removal of one adrenal the rise was not as great (163 per cent). A smaller rise was observed following excision of the second adrenal (81 per cent above basal of a double adrenalectomized animal or 38 per cent lower than the metabolism of a normal rat measured at 4°C.).

*Is the adrenal X zone andromimetic?* EVELYN HOWARD. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

The X zone of the mouse adrenal hypertrophies following removal of the testes. This suggests that this tissue may be a source of material which stimulates male accessory structures, an effect known to be associated with adrenal tumors, which may be described as andromimetic. This conception would account for certain effects of tumors and also explain failures to obtain consistent sex modifying action with extracts of apparently non-X zone bearing cortex.

Preliminary to studying the effects of X zone extracts on male accessories, I have compared the effects of castration in rats and mice. In contrast to mice, castrated rats do not ordinarily develop an X zone. In accord with others, I find that the epithelium of the seminal vesicles and prostate of the adult rat degenerates very markedly within twenty days after castration. In the X zone bearing mouse, on the contrary, although castration results in considerable reduction in the gross size of these structures, and some histological alteration, nevertheless much of the epithelium (both prostate and vesicles) retains its high columnar character and a fairly normal general appearance, for as long as forty days of castration. In rats castrated at birth Price has described a marked subsequent but transitory development of the prostate. I have confirmed this observation and also find that the prostate development is accompanied by cellular enlargement and other changes in the inner part of the adrenal cortex.

Regarding effects of the X zone on ovarian function, I have compared normal X zone bearing female mice with adrenalectomized females maintained on extracts of non-X zone bearing glands. Growth of the adrenalectomized mice was maintained at a practically normal level with such an extract, whereas untreated adrenalectomized mice showed inhibition of growth and died within a few days. The presence of X zone did not retard oestrus or affect fertility.

These observations are consistent with the hypothesis that the X zone exerts an andromimetic effect.

*Subtotal ligation of the arteries to the liver.* CHARLES HUGGINS and JOSEPH POST (by invitation). The University of Chicago, Chicago, Ill.

We have confirmed previous observations on dogs that ligation of the hepatic artery prior to its anastomosis, *a*, with its principal collaterals at the porta hepatis is without effect and that ligation beyond this point is invariably fatal. It was found, however, that the entire hepatic artery could be removed from its source to deep in the liver some days following preliminary ligation at *a*. This multiple stage ligation is well borne and no changes were detected in the liver, even following subsequent bilateral phrenic artery ligation: in these preparations the arterial circulation time was greatly slowed and portal vein oxygen content was much reduced in passage through the liver.

*A photocell multimorph stimulator.* I. F. HUMMON, JR. (introduced by T. E. Boyd). Loyola University School of Medicine, Chicago, Ill. (Demonstration.)

The construction of the stimulator provides for 1, a stationary wave-form mask; 2, means for passing a very narrow beam of light over this mask at variable speeds; 3, lenses to throw this beam of light upon photocells, and 4, other accessory equipment. Because the wave-form mask is stationary it may be varied during stimulation. Two photocells are used which make it possible to produce true alternating currents. A variable speed drive controlling the passage of the beam of light over the mask provides for a frequency variation from 1 per second to 1500 per second. This range may be greatly extended by the use of optical multipliers.

Special provision may be made for producing two consecutive stimuli which are independently variable as to wave-form, duration, intensity,

and polarity. Also the interval between the stimuli may be varied from zero to a predetermined maximum while the equipment is in operation.

*On the relation between hypoglycemia and anoxemia.* R. C. INGRAHAM (by invitation), L. F. MOLDAVSKY (by invitation) and E. GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago.

It was attempted to clarify the relationship between anoxemia and hypoglycemia by blood pressure studies in which the blood pressure response of the anesthetized dog to  $O_2$  deficiency was determined at various blood sugar levels. The experiments were carried out on 30 dogs narcotized with sodium barbital or sodium amytal. Air with 6.2 per cent  $O_2$  was inhaled from Douglas bags for 3 minutes. Insulin (Lilly) was injected intravenously and blood sugar, pH, phosphorus, potassium and calcium in the blood were determined during the course of the insulin hypoglycemia.

It was found that the rise in blood pressure occurring during inhalation of 6.2 per cent  $O_2$  for 3 minutes increases as the blood sugar falls. Intravenous injection of glucose, which offsets to a certain extent the hypoglycemia induced by insulin, diminishes the blood pressure response to  $O_2$  deficiency.

*The effects of thyroidectomy, castration, anterior lobe administration and pregnancy upon experimental diabetes insipidus in the cat.* W. R. INGRAM and C. FISHER (by invitation). Department of Anatomy, State University of Iowa, and Institute of Neurology, Northwestern University, Evanston, Ill.

The observation previously made, that thyroidectomy may somewhat reduce but does not abolish the polyuria produced by lesions of the supra-optico-hypophyseal system in cats, has been confirmed in 4 other cats. Feeding desiccated thyroid to such animals elevates the water exchange to equal or exceed the level existing before thyroidectomy.

Castration performed on 4 cats with diabetes insipidus failed to diminish the polyuria, and in one of these the water exchange increased after ovariectomy.

Administration of a crude anterior lobe preparation, while ineffective in producing polyuria in normal cats, may increase the water exchange in cats with a tendency to diabetes insipidus. It is not invariably effective, however, and cats already displaying marked polyuria may not respond to such treatment.

Cats operated in the early stages of pregnancy may develop marked polyuria which persists, with some indication of diminution immediately before parturition. In the few cases available at present, parturition has been apparently abnormal, has not been completed and has ended fatally for the mother cat. This has prevented observation as to the course of the polyuria after parturition.

*Differential vascular changes during apnea from inflation of the lungs.*

LAURENCE IRVING. Department of Biology, University of Toronto, Canada.

During inflation of the lungs and the resulting apnea, the flow of blood through the muscles diminished and the flow increased through the brain. Changes in blood flow were determined by the cooling effect which the cir-

ulation exerted upon a resistance wire which was heated by a constant electrical current.

The change in blood flow was observed to start about 5 seconds after the apnea from lung inflation began, and it continued as long as the apnea lasted. Following the apnea, recovery changes occurred, with increased flow in both muscle and brain. The increased blood flow in the brain during apnea occurred in spite of a fall in blood pressure, and did not depend upon blood pressure changes. The decreased muscular flow likewise did not agree with pressure changes, and failed to occur in the hind leg after denervation.

This differential vascular change was first observed in beaver and was regarded as a respiratory adjustment facilitating the endurance of asphyxia. The same differential vascular reaction is present, however, in rabbits and cats, although it is less conspicuous. Knowing that the oxygen capacity of all mammals is similar, it is suggested that the adequacy of circulatory adjustments helps to provide the variations which mammals show in their ability to resist asphyxia.

*On the hypnotoxin theory of sleep.* A. C. IVY and J. G. SCHNEDORF (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

The hypnotoxin theory of sleep was advanced by Pieron (1913) in his monograph. His best point of evidence was obtained by removing cerebrospinal fluid from a fatigued animal and injecting it intraventricularly into a normal animal. The injected animal became drowsy and fell "asleep." We have kept twelve animals awake in the standing position for seven or more days. Eight centimeters of cerebrospinal fluid were withdrawn aseptically by cisternal puncture from normal dogs and replaced by 8 cc. of fluid from the sleep-deprived dogs (fatigue fluid). In from 15 to 30 minutes the dogs became drowsy, and were markedly depressed in from 1 to 2 hours after the injection. Three of the twelve could be aroused only with difficulty. The animals remained depressed for from 5 to 8 hours, when complete recovery occurred. Among other observations, we found that the body temperature of the dogs receiving the "fatigue fluid" rose on the average of 2.6°F. (no panting) and that the rise and fall in temperature corresponded to the onset and remission of the depression. (The depression was not due to hemorrhage, since out of 120 cisternal punctures blood tinged fluid was obtained only four times.) It was found that the simple withdrawal of fluid from a normal animal does not cause an elevation in temperature; but, the withdrawal of 8 cc. of fluid from a normal animal into a syringe (kept warm) and its reinjection into the same animal, caused an average rise in temperature of 2.0°F. (The fluid is withdrawn in 3 min. and reinjected in 3 min.) These animals became depressed. Of 42 control animals receiving normal fluid or sodium chloride all showed an elevation of temperature and all but 2 were depressed. However, none of them became as depressed as 3 of the 12 animals which received "fatigue fluid." Thus, we have confirmed Pieron's observation, but cannot confirm his interpretation unequivocally until a way is found to administer the "fatigue fluid" without causing a significant rise in body temperature.

*The electrocardiogram of the grasshopper.* THEODORE LOUIS JAHN and FREDERICK CRESCITELLI (introduced by J. H. Bodine). State University of Iowa, Iowa City. (Read by title.)

Using a cathode ray oscillograph and a condenser-coupled amplifier, a study has been made of the electric potentials associated with the beating heart of the grasshopper (*Melanoplus differentialis*). A complex diphasic wave which shows definite indications of oscillatory activity has been found to be the typical form of electrocardiogram.

With the "exploratory" electrode on the heart and the "indifferent" electrode either in the surrounding fluid medium or on the neighboring "inactive" tissues it has been found that the general form of the wave does not change 1, when the location of the "indifferent" electrode is changed, and 2, when the location of the "exploratory" electrode is altered. A diphasic wave with the positive potential phase (with respect to the "exploratory" electrode) always occurring first has been found to be characteristic for this type of experiment. True monophasic waves have not been recorded when only one electrode is on the heart.

If two electrodes are led off from the heart the wave form of the electrocardiogram is not significantly different from that obtained with only one cardiac lead. In many experiments identical wave forms have been photographed with either one or two electrodes placed on the heart. In these experiments with two cardiac leads, however, the sign of the initial phase may be positive or negative depending, apparently, on the position of the two electrodes with respect to the spread of the excitatory process in the heart. The polarity of the diphasic wave for a given heart has often been reversed by merely altering the longitudinal location of the two electrodes along the heart. It has not been possible to relate this reversal of phases to any particular morphological point or region of the heart.

*On the mechanism of block as produced by ouabain in the isolated embryonic heart.* J. RAYMOND JOHNSON and GEORGE H. PAFF (introduced by G. B. Ray). Departments of Physiology and Anatomy, Long Island College of Medicine, Brooklyn, N. Y.

When isolated hearts of 48 hour chick embryos are exposed to a solution of ouabain one of the first noticeable effects is the appearance of a partial block between the sino-atrium and ventricle. The degree of block becomes progressively greater until the ventricle stops completely. Later the sino-atrium also stops. In order to determine the possible mechanism of this block the behavior of hearts has been studied both microscopically and photographically.

In hearts which have stopped beating under the influence of ouabain strong stimulation fails to elicit a response. These same hearts, after washing in Tyrode solution, respond to electrical and mechanical stimuli and eventually return to the original state of inherent spontaneous contractions. The effect of the ouabain is one of depression of irritability in the entire heart. Since in normally beating hearts the sino-atrium and ventricle may be completely divided and each part continue to beat with its own rhythm, the block with ouabain is not due merely to failure of impulse conduction between the two parts but is an expression of depressed irritability as well. The degree of effect depends upon the inherent activity of the particular portion of the heart.

*Studies on the dynamics of the pulmonary circulation.* VICTOR JOHNSON, W. F. HAMILTON, L. N. KATZ and W. WEINSTEIN (by invitation). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago; Department of Physiology, University of Chicago, and Department of Physiology and Pharmacology, University of Georgia, Augusta.

With the Hamilton technique for recording blood pressure, studies were made upon the pulmonary circulation of lightly anesthetized dogs. A four inch incision between adjacent ribs provided ample room to isolate and cannulate various pulmonary vessels. Closing the incision tightly about the lead tubes and aspirating the air from the chest permitted most of the studies to be made on animals breathing normally. Hemorrhage was minimal and shock infrequent as attested by the high systemic arterial pressures.

The respiratory changes in intrathoracic pressure produced parallel changes in both aortic and pulmonary arterial pressures. All three curves, recorded simultaneously, varied together. The absence of lag in the pulmonary arterial pressure fluctuations suggests that the intrathoracic pressure changes affect the pulmonary vessels directly.

Marked rises in the systemic arterial pressure induced by partially occluding the aorta failed to induce appreciable changes in the pulmonary arterial pressure, presumably because of the large capacity of the pulmonary bed and the low elasticity coefficient of the pulmonary vessels. These findings in animals breathing normally confirm results reported on dogs with pneumothorax and artificial respiration.

The velocity of the pulmonary pulse wave was estimated by placing one cannula in a central artery and another in a peripheral artery a measured distance away and noting the time interval elapsing between the rise of the pulse curve at these two points. The velocity was found to be about 3.5 meters/second. The apparent linear velocity was less in deflated than in inflated lungs, and, of course, it was less at lower pulmonary arterial diastolic pressures than at high pressures.

Adrenalin injections yielded results difficult to interpret. The aortic pressure rose, and the jugular pressure fell, but simultaneously both the arterial and venous pulmonary pressures rose. This suggests that pulmonary congestion is an important causal factor in producing the changes. Pulmonary vasoconstriction was not ruled out.

*The effect of intravenous injection of sodium hexa meta phosphate on the dog's heart.* K. K. JONES and D. E. MURRAY (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Behrens and Seelkopf (Arch. f. Exper. Path. u. Pharmacol. 169: 238, 1933), observed the toxic effect of pyro phosphates, and the tri and hexa meta phosphate on dogs, and suggested that this was due to a precipitation of calcium in the blood by these phosphates.

On repeating and extending this work the authors have shown that the action of the complex sodium hexa meta phosphate on the heart is due to its effect on the calcium of the blood.

It was found that when the minimum amount of 138 mgm. per kilo was injected intravenously, the blood pressure fell to zero, the heart stopped beating in diastole and could not be revived. With smaller doses of the hexa meta phosphate, less severe reactions occurred and the heart

would survive the drop and in five minutes or less return to a normal indistinguishable from the condition before injection.

With decreasing doses the effect noted on the heart became less and less until at a dose of 20 mgm. per kilo, no drop in blood pressure occurred, but merely a change in heart beat for a short time to the vagal type was noted.

This effect of the hexa meta phosphate on the heart could be completely removed by the injection into the blood stream of  $\text{CaCl}_2$  in the ratio of 1 mgm.  $\text{CaCl}_2$  to 1.33 mgm. of the hexa meta phosphate. There was no marked effect even on injecting lethal doses of either  $\text{CaCl}_2$  or the meta phosphate providing the other was injected at the same time in carefully balanced amount. The immediate return to normal following such injections and the entire lack of injury of repeated injections of sub lethal doses indicated that no toxic action was caused other than the effect on the ionized calcium of the blood.

No injury of the red blood cells was noted in this procedure, but a transient increase in bleeding time of from five to ten minutes was noted following the injection of the hexa meta phosphate.

*Further observations on the subareolar node.* F. T. JUNG and A. L. SHAFTON (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

In a previous communication we gave a chronology of some physical changes of puberty with reference to the appearance of the subareolar node. This is a physiological tumor which consists of glandular and connective tissue; it becomes palpable under each nipple in the midst of the pubertal changes. Our first results were based on a single examination of each of a limited number of boys. We are now able to add data obtained from several other groups, bringing the total number of individuals examined to more than 700. Of these, 155 have been seen 3 times; i.e., they have been re-examined 6 and 15 months after their first examination.

Some of our conclusions are as follows: Probably every boy at some time between the ages of 12 and 18 shows a mass under each nipple. The right and left nodes do not always appear at the same time. They reach a maximum size in a few months, and maintain it (but with fluctuations) for about half a year. A node at this stage is likely to have a diameter of 1.5 cm., and is spherical and hard; but after this stage there is a great diversity of behavior. Some nodes become much larger; a few go on to a definite, but probably temporary gynecomastia. The largest glandular masses so far seen by us in sparely-built and apparently normal subjects measured *a*, 6.2 by 6.9 cm. in a boy of 14.5 years, and *b*, 5.6 by 6.0 cm. in a youth of 18.5 years. (Subjects with marked obesity and other confusing features are excluded here.) Most nodes decline slowly; some disappear within 3 years. Of 84 men between 20.00 and 20.99 years of age, 12 still had palpable nodes.

*An analysis of cardio-accelerator fibers in the vago-sympathetic trunk of the dog.* HERMAN KABAT (introduced by M. B. Visscher). Department of Physiology, University of Minnesota, Minneapolis.

Stimulation of the dog's vago-sympathetic nerve after atropine causes acceleration of the heart. Experiments were carried out to determine whether the accelerator fibers are true vagus fibers or sympathetic fibers that join the nerve in the neck.

In dogs under chloralose anesthesia, cardiac acceleration was never

observed in response to stimulation of the vagus rootlets intracranially. Intense cardiac inhibition was elicited from the bulbar accessory and lower vagus rootlets while stimulation of the upper vagus rootlets had no effect on the heart rate.

In other experiments, the spinal cord was cut at C2, atropine administered and the dog maintained by artificial respiration. Stimulation of the bulbar accessory or vagus rootlets intracranially, even with a strong faradic current, had no effect on the heart rate, though marked rotation of the head occurred as a result of spread of current to the spinal accessory nerve. Slight acceleration occurred in one experiment during stimulation of the vagus rootlets with a strong current. Stimulation of the vago-sympathetic trunk with weaker currents provoked marked cardiac acceleration. With both vagi intact, sudden anemia of the brain caused no reflex acceleration. Stimulation of the central end of one vagus with the opposite vagus intact had no effect on the heart rate.

More marked responses were obtained by stimulating the vago-sympathetic low in the neck than in the mid-cervical region and the right nerve was more effective in accelerating the heart than the left. Acceleration occurred after a fairly long latent period and the effect persisted for some time after cessation of stimulation.

It appears, therefore, that the acceleration resulting from stimulation of the vago-sympathetic trunk after atropine is dependent largely, if not entirely, on the sympathetic cardiac nerves which run in the trunk. If any true vagal accelerator fibers are present, they play no significant part in reflex adjustment of the heart rate.

*The rôle of the liver in regulating the distribution and rate of the blood flow.*

L. N. KATZ and S. RODBARD (by invitation). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, Ill.

While the importance of the splanchnic bed in regulating the distribution and rate of the blood flow is well known, the part played by the liver blood vessels in these adjustments is still not well understood. As a first step in investigating the rôle of the liver, we have carried out a series of acute experiments on anesthetized dogs in which arterial, portal and venous pressures were recorded simultaneously with the portal blood flow following the use of several drugs.

Ringer's solution increased the portal flow. Adrenalin, amyl nitrate, acetylcholine, mecholyl, histamine and digitalis all caused a decreased flow in the portal vein, but the action of these drugs on the pressures were different in each instance. The results are summarized in the following table.

DRUGS	ARTERIAL PRESSURE	VENOUS PRESSURE	PORTAL PRESSURE	PORTAL FLOW
Adrenalin.....	+ then -	+ then -	+	- then +
Mecholyl.....	-	-	- then +	-
Digitalis.....	+	-	+	-
Histamine.....	-	+ then -	+	-
Amyl nitrite.....	-	0	-	-
Ringer's transfusion.....	+	+	+	+

These diverse changes in the pressures indicate that the blood vessels of the liver react differently to these various drugs, and by their changes

in caliber significantly modify the flow of blood through the splanchnic area. The liver is the portal of exit of the splanchnic bed, a portal which can be varied in size. Such changes in liver vascular resistance will help to influence the amount of venous return and also the way in which the blood flow is distributed. The capacity of the splanchnic bed and the flow within it, on the basis of this work, is dependent not only on the tone of the splanchnic blood vessels themselves but also on the tone of the liver blood vessels. This study gives no indication of the particular part of the vascular tree of the liver which plays the dominant rôle.

*Differential reflex effect of chemical carotid gland stimulation on reflexes of respiratory and non-respiratory somatic musculature.* WILLIAM KAUFMAN (introduced by Robert Gesell). University of Michigan, Ann Arbor.

The effects of chemical stimulation of the carotid gland on respiration and on reflex contraction of the tibialis anticus muscle were studied in morphine-urethane anesthetized dogs. The tibialis anticus muscle was reflexly activated by induction shocks of constant strength and rate. Uniformly, in all the animals studied there was a hyperpnea following the chemical stimulation of the carotid gland which in most animals (90 per cent) was accompanied by varying degrees of inhibition of the tibialis anticus muscle. This inhibition began simultaneously with the hyperpnea and lasted as long as the hyperpnea. The interpretation of these findings is that chemical stimulation of the carotid gland exerts reflexly a differential effect on reflexes of respiratory musculature and on reflexes of non-respiratory musculature. This reflex arrangement may favor respiratory adjustments in time of need.

*The effect of certain potassium salts on acid base excretion in the normal individual.* N. M. KEITH, A. E. OSTERBERG (by invitation), and M. W. BINGER (by invitation). Mayo Clinic, Rochester, Minn.

Several observers have demonstrated that potassium salts administered orally in relatively large amounts may exert no toxic effect. Since potassium is readily excreted by the kidney it seemed important to study its action on the rates of excretion of acid and basic ions in the normal individual.

For these experiments voided specimens were collected hourly for a nine hour period during the day while fasting. A total of 600 cc. of water was ingested at regular intervals during the nine hour period. Venous blood samples were withdrawn at repeated intervals. Control observations included two experiments in which no salt was ingested and three experiments in which sodium bicarbonate was given. Potassium salts ingested were the bicarbonate, chloride and nitrate in doses of 0.11 to 0.16 gram per kgm. of body weight in a 25 per cent solution at the beginning of the second hour. Each potassium salt produced an increase in renal excretion of water and of chloride, bicarbonate, sodium, and potassium ions. After the ingestion of potassium bicarbonate the urine pH rose to 7.9 and the bicarbonate excretion was markedly increased. These conditions continued for several hours. Potassium chloride caused a rise in pH to 7.7 and a moderate increase in bicarbonate excretion although the alkaline urine only persisted for three to four hours. Potassium nitrate caused an initial shift of the urine pH to 7.1 and an increase in bicarbonate ion, but

in four hours the pH had fallen to 5.0 and bicarbonate ion to a trace. This latter result suggests that the kidney was excreting the nitrate and potassium ions in varying proportions during different periods of the experiment. In these studies potassium salts caused only slight shifts in the concentration of bicarbonate in the plasma, however, they gave rise to marked changes both in bicarbonate and hydrogen ion concentrations in the urine. The physiologic action of potassium salts in the kidney differs in many respects from that of sodium.

*Degeneration of the infundibular nerve fibers in the cat without eliciting diabetes insipidus.*<sup>1</sup> A. D. KELLER and J. W. HAMILTON, JR. (by invitation).

Department of Physiology and Pharmacology, University of Alabama.

Healthy cats that had been conditioned to our cage regime were used. Attempts were made to sever nerve fibers that passed from the hypothalamus to the hypophysis by way of the infundibulum as they originate and course in the ventral hypothalamus. Lesions were placed by subpial manipulation with the blunt end of a milliner's needle. The extent of the lesion in the hypothalamus and the degree of nerve fiber degeneration in the pars nervosa were determined by sectioning the hypophysis and hypothalamus longitudinally and staining appropriate sections for nerve fibers by Bodian's method. Water was available continuously while food, consisting of Balaration baited with salmon, was available for sixteen hours daily. The animals' post-operative courses were uneventful and normal, food and water being consumed spontaneously. Duration of experiments ranged from five to thirteen weeks.

The series consisted of seventeen cats. Complete degeneration of the nerve fibers in the pars nervosa occurred in seven instances. One of these animals exhibited a typical diabetes insipidus of moderate severity. Her water consumption, with normal food intake, for twenty-four hour periods ranged from 300 to 550 cc. per kilogram body weight. In two other cases, of this group, a trace of diabetes insipidus was suspected, while the four remaining animals were unquestionably negative. The pars nervosa was essentially denervated in six other cats, since only occasional nerve fibers remained. Possible traces of diabetes insipidus was present in three of these animals; the others were negative. Denervation was incomplete in the remaining four attempts, one of which exhibited a trace of diabetes insipidus; the others being negative.

It is apparent that in this series the presence or severity of diabetes insipidus could not be correlated with denervation or the degree of denervation of the pars nervosa, i.e., the infundibular process plus the infundibulum, which in turn includes the median eminence or eminentia sacularis.

*Hemi-ablation of the cerebellum in the monkey without irregularity in voluntary muscular movement.*<sup>2</sup> A. D. KELLER, W. P. CHASE (by invitation) and R. S. ROY (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Motion pictures.)

Unilateral decerebellization was accomplished by the simultaneous extirpation of the whole of the vermis and the left lateral lobe. The cortex of the right lateral lobe with its underlying lateral cerebellar nuclei and the right brachium conjunctivum remained intact.

<sup>1</sup> Aided by a grant from the Rockefeller Foundation.

<sup>2</sup> Aided by a grant from the Rockefeller Foundation.

During the acute stage following operation there was present a postural deficit characterized by an impairment of balance and awkwardness in movements of progression. In the final state, balance seemed unimpaired. It was suspected, however, that in progression there remained a slight lack of smoothness—tendency to cerebellar gait—in muscular movement. The lack of asymmetry, in such a preparation, either during the acute or final state is evidence that this deficit is due to ablation of the vermis alone. This was also verified by extirpation of the vermis leaving both lateral lobes intact. The vermal cortex seems not involved.

Except for a slight awkwardness in manipulating food for the first day or two after operation—presumably due to the postural deficit—there was no disturbance in voluntary muscular movement as evidenced by the absence of irregularity in feeding movements, picking over the skin, catching flies, etc. Spontaneous and postural tremors were absent directly following operation but frequently appeared transitorily with varied severity and duration several days after operation. These symptoms appeared as frequently in the right limbs as in the left limbs in spite of the intactness of the right lateral lobe. This fact, as well as the lateness of onset of the tremors indicate that they are due to delayed transitory derangement of brainstem structures rather than being caused by the cerebellar ablation.

It is, therefore, evident that the cerebellum, in its entirety, is not essential for the ordinary coördination of voluntary muscular movement nor for muscular stability when at rest or during movement.

*Normal sex functions following section of the hypophyseal stalk in the dog.<sup>1</sup>*

A. D. KELLER and J. W. HAMILTON, JR. (by invitation). Department of Physiology and Pharmacology, University of Alabama. (Read by title.)

In view of the frequent association of genital atrophy with tumors or injury in the hypothalamic region and also because of the remote possibility that the infundibular nerve fibers might be an efferent pathway involved in the mechanism of ovulation, it seemed worthwhile investigating sex functions following separation of the hypophysis from the hypothalamus.

To date, two dogs having the hypophyseal stalk sectioned with scissors have, subsequent to operation, bred and whelped normally litters of normal pups. In one case coitus was observed on the 168th day after operation and an uneventful delivery of 3 pups occurred 62 days later. Lactation appeared normal, but the pups died from exposure during the 1st week. The line of section of the stalk was such that a considerable portion of the pars tuberalis remained attached to the brain. However, the greater part of the infundibulum, the whole of the fundibular process, pars anterior and pars intermedius were separated. In the other instance, coitus was first observed on the 122nd day after operation and an uneventful delivery of 4 pups occurred 62 days later. This dog did not lactate and the pups died for lack of food. The stalk in this instance was separated such that only a fragment of the anterior portion of the pars tuberalis remained attached to the brain. A proximal ventral fragment of the infundibulum (median eminence) likewise was not separated. There was some atrophy of the anterior portion of the pars anterior.

<sup>1</sup> Aided by a grant from the Rockefeller Foundation.

Another dog in which the entire hypophysis was isolated from the hypothalamus by a rather massive involvement of the ventral portion of the hypothalamus, subsequent to operation, bred and whelped normally a litter of 3 pups. This case is of particular interest because there was present a diabetes insipidus of moderate severity (600 cc. of water per kilogram body weight per day) which appeared promptly after operation and persisted until the animal was terminated ten months later.

*Forced circling movements in monkeys following lesions of the frontal lobe.*

MARGARET A. KENNARD and LEON ECTORS<sup>1</sup> (by invitation). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Primates from which one cerebral hemisphere has been removed circle, in walking, toward the side of the lesion. This circling appears to be automatic, i.e., "forced" in character. The performance of almost any motor act precipitates such circling, and any affective stimulus such as fright or the sight of food induces it in exaggerated form. In monkeys (*Macaca mulatta*) forced rotatory movements occur only in animals which show turning of the head and conjugate deviation of the eyes toward the side of the lesion. Extirpation of an area in the mesial frontal convolution in the region of the superior limb of the arcuate sulcus (including part of area 8) gives rise to these adverse postures. Following unilateral lesions the duration of the head and eye turning is transitory, lasting only a few weeks, but circling may occur for several months thereafter. Extirpation of the occipital lobe, or of the motor face area is not followed by circling movements, nor are extirpations of any or all other areas of the frontal lobe, provided this small region remains intact.

Increased motor activity following bilateral extirpations of frontal lobes has been reported by Richter and others. The forced character of the movements seen following lesions affecting area 8 suggests that removal of this region of the frontal lobe may be responsible for such increased activity.

*The effect of double vagotomy and carotid sinus denervation on the reaction of barbitalised dogs to hyperventilation.* C. E. KING. Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tenn.

More than one hundred experiments have yielded conclusive evidence that double vagotomy in the mid cervical region in barbitalised dogs does not demonstrably alter the period of apnea following hyperventilation. This indicates clearly that there are no vagal receptors below the level of section which are affected by the chemical changes taking place in the blood during the apneic period.

The picture at the beginning of the hyperventilation period however is altered. Before the nerves are sectioned, there is an immediate cessation of spontaneous breathing on part of the animal, at least as judged by visible evidence and mechanograms from the chest. After vagotomy spontaneous respiratory efforts occur until the acapnia has become quite pronounced. The rate of these efforts is identical with the rate of breathing before the ventilation is started. It is surmised that before vagotomy

<sup>1</sup> Fellow of Commission for Relief of Belgium Educational Foundation.

spontaneous respiratory movements cease entirely upon the incidence of hyperventilation, or that they persist for some time but fall in phase with the artificial ventilation.

In contrast to double vagotomy, denervation of the carotid sinuses produces no effect upon the initial phase of hyperventilation, but causes a greatly prolonged period of apnea. Our standard procedure was to artificially ventilate the animal for three minutes so as to approximately quadruple the minute volume of air. In the control experiments the average duration of the period of apnea was 90 seconds; after denervation of the sinuses more than half of the animals showed no signs of spontaneous recovery of breathing, and in those instances in which spontaneous recovery took place the apneic periods were prolonged far beyond any observed in the control experiments. Resuscitation proved difficult. Oxygen alone was generally not effective, but a combination of oxygen and a high percentage of carbon dioxide yielded better results.

Our results fall in line with the concept that receptors in the region of the carotid sinuses are of prime importance in the respiratory reactions to chemical changes in the blood, in particular those associated with anoxemia.

*The carotid sinuses in relation to splenic contractions induced by anoxemia.*

C. E. KING. Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tenn. (Read by title.)

It is well established that receptors located in the region of the carotid sinuses play an important part in the reaction of the respiratory mechanism to anoxemia. This inquiry has as its objective the determination of the rôle, if any, of these receptors in the reactions of the spleen to anoxia.

The experiments were done on fifteen dogs, anesthetized with barbital-sodium administered intravenously. The animals so prepared presented conditions quite favorable for such studies, inasmuch as the spleen became engorged with blood with the advent of anesthesia, but remained quite reactive to the various procedures which ordinarily induces splenic contractions, among them anoxemia. Blood pressure records were obtained from a femoral artery and the splenic contractions were recorded by means of an oncometer and tambour system.

In the control experiments the animals were made to breathe from a Tissot spirometer a gas mixture containing 90 per cent  $N_2$ , 7 per cent  $O_2$  and 3 per cent  $CO_2$ . The respiratory reaction was, in practically all instances, quite pronounced. The  $CO_2$  was added because we have learned that acapnia itself frequently induces splenic contractions. Splenic contractions were consistently obtained. The rate varied somewhat between different animals, but ranged between one and two contractions a minute. The carotid sinuses were then denervated by dissection and painting with a 5 per cent novocain solution. After allowing time for the spleen to refill, the animals were made to breathe a mixture of 7 per cent  $O_2$  in  $N_2$ . The  $CO_2$  was omitted because hyperventilation did not occur, consequently no acapnia developed. The splenic contractions were not altered either in rate or depth.

Our results indicate that splenic contractions induced by anoxia are neither mediated nor measurably influenced through receptors lying in the carotid sinuses.

*Some factors influencing thermal panting in cats.* FRANÇOIS KLEYNTJENS<sup>1</sup> (introduced by Philip Bard). Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

Thermal panting in cats is characterized by open mouth and movements of tongue and labial commissures synchronous with the rapid respiratory excursions. Normal cats apparently never pant in response to environmental warmth unless deep rectal temperature rises. In twenty experiments the rise averaged  $0.8^{\circ}\text{C}$ .; its range was  $0.2^{\circ}$  to  $1.9^{\circ}\text{C}$ . Under ether or urethane cats pant when a greater rise is induced.

An anesthetized cat with rectal temperature raised to a point below that requisite for spontaneous panting immediately pants when its jaws are passively opened. This response is apparently dependent on proprioceptive impulses from the masticatory muscles; it cannot be evoked by stimulation of buccal mucosa or tongue and is abolished by bilateral section of the third division of the fifth nerve. Following such section normal panting, although less well sustained than in the intact animal, begins if the rectal temperature rises above the critical level. The response to opening the mouth can be similarly elicited in the unanesthetized normal cat.

Although an increase in the temperature of the skin or of the air breathed cannot evoke panting, each of these changes exerts an influence once the rectal temperature has been raised. Typical thermal panting can occur after double vagotomy and spinal transection at the sixth cervical segment. After section of the phrenics such a preparation, maintained by artificial respiration continues to show the characteristic tongue and lip movements.

Chronically decorticate cats show a normal capacity to pant. Nevertheless, panting can be modified by impulses of cortical origin. In the intact anesthetized cat electrical stimulation of a small cortical area just ventral to the rostral extremity of the coronal sulcus inhibits all panting movements. The inhibitory effect is followed by a rebound which involves only the face and tongue movements. Unilateral stimulation exerts bilateral effects and removal of the opposite frontal pole does not modify this result.

*"Psychic blindness" and other symptoms following bilateral temporal lobectomy in Rhesus monkeys.* HEINRICH KLÜVER and PAUL C. BUCY. Otho S. A. Sprague Memorial Institute and Divisions of Psychiatry, Neurology and Neurosurgery, University of Chicago, Chicago, Ill.

In connection with a study of hallucination-producing drugs the temporal lobes of an adult Rhesus monkey were removed in two stages. Up to the present (4 weeks after removal of the second lobe) the following symptoms can be observed. The animal does not exhibit the reactions generally associated with anger and fear. It approaches humans and animals, animate as well as inanimate objects without hesitation and although there are no motor defects, tends to examine them by mouth rather than by the use of the hands. There is a general slowing down of movements; the quick, jerky movements characteristic of the normal Rhesus monkey have almost entirely disappeared. Various tests do not show any impairment in visual acuity or in the ability to localize visually the position of objects in space. However, the monkey seems to be unable

<sup>1</sup> Fellow of the C. R. B. Educational Foundation.

to recognize objects by the sense of sight. The hungry animal, if confronted with a variety of objects, will, for example, indiscriminately pick up a comb, a bakelite knob, a sunflower seed, a screw, a stick, a piece of apple, a live snake, a piece of banana, and a live rat. Each object is transferred to the mouth and then discarded if not edible. In Klüver's "formboard" test the same response is made to the stimulus figures, such as a square or a circle, as to the food itself. In each test the animal transfers first the piece of cardboard with the stimulus figure to the mouth, examines it and then does the same with the food. These symptoms of what appears to be "psychic blindness" are not present in four other monkeys which are being studied at present and in which only one temporal lobe has been removed. However, these cases seem to respond less easily and less strongly to a variety of stimuli which in monkeys with one or both frontal, parietal or occipital lobes extirpated or in normal monkeys, call forth extreme excitement as evidenced by motor or vocal behavior.

*The contractile element of the acetylcholine contracture of denervated muscle.*

G. CLINTON KNOWLTON (by invitation) and H. M. HINES. Department of Physiology, State University of Iowa, Iowa City.

Isotonic and isometric responses of rats' denervated gastrocnemii have been studied: 1, to remote intravenous injections of acetylcholine under circumstances in which the normal muscle is not stimulated by acetylcholine; 2, to adjacent intra-arterial injections of acetylcholine under circumstances in which the normal muscle does respond to acetylcholine injection, and 3, to direct tetanic stimulation. These responses have been compared with those of rats' normal gastrocnemii to acetylcholine injections, and to direct stimulation. It is concluded, from these results, that the contractile element for the acetylcholine contracture of denervated muscle is not the normal straight fiber.

*Animal experiments on normal and pathological sound conduction.* H. G.

KOBRAK and H. G. PERLMAN (introduced by A. J. Carlson). University of Chicago, Chicago, Ill.

Animal experiments were carried out for the purpose of studying the pathways of sound conduction under normal and pathological conditions. We already possess information regarding the normal pathways. In this study an attempt is being made to observe the action of substitute-pathways after destruction of the normal sound conduction apparatus. The acoustic reflex of the muscles of the middle ear was used in the rabbit as indicator of cochlear function.

First the normal proportion between ossicular and aerocochlear sound conduction was determined by the following method:

The cochlear irritation by an air-conducted sound is determined with the bulla ossea wide open at its ventral side. The procedure is repeated after the bulla opening is sealed so that no sound energy can enter the middle ear cavity directly. The sealing has been found to be of no effect, indicating that the aero-cochlear conduction is negligible under normal conditions. The same experiment has been carried out after artificial impairment of the normal sound conduction by plugging the outer acoustic canal. The amount of sound intensity now entering the cochlear by aero-cochlear conduction is no longer negligible. The sealing of the bulla

opening diminishes the reflex contractions considerably. A normally unimportant pathway has become an essential sound conductor in deafness. Its elimination, therefore, increases the deafness.

In another series of experiments the conduction of sound through the bone was tested. In air conduction the part of sound energy which travels through the bone is negligible. After destruction of the ossicles an air conducted sound may under certain conditions stimulate the cochlea so strongly by bone conduction, that the middle ear muscle reflex is elicited.

A bone conducted sound (receiver applied to teeth) travels mainly through the bone of the skull. Destruction of the ossicular chain does not diminish the tensor reflex induced by bone conduction. On the contrary there is a slight real increase of bone conduction demonstrable after destruction of the ossicular chain.

The intralabyrinthine fluid was studied to determine its importance for the three main pathways of sound. The loss of function for ossicular, aero-cochlear and bone conduction is uniform, if one removes the labyrinthine fluid. The Inner Ear liquid, therefore, seems to be a part of all pathways, and could be called the "final common path" for all sound routes. A direct irritation through the bone could not be demonstrated.

*The comparative efficacy of various androgens as determined by the rat assay method.* CHARLES D. KOCHAKIAN (introduced by J. R. Murlin). Department of Vital Economics, University of Rochester, Rochester, N. Y.

Rats castrated at 70 to 90 grams were injected 30 days later for 6 days and on the seventh day killed and the accessory sex glands removed. The organs were fixed in 10 per cent formalin for 24 hours, transferred to 70 per cent alcohol, cleaned, and the seminal vesicles separated from the prostate. Weighings were made on a torsion balance after drying the organs between paper towels. Control rats showed an average value of 6 mgm. for the seminal vesicles and 35 mgm. for the prostate.

All of the preparations used were dissolved in the same lot of olive oil and so that each injection would be contained in 0.3 cc. of solution.

Taking testosterone as unity a comparison of the effect on the seminal vesicles and prostates of 0.24 mgm. per day of the following androgens was: androstenediolbenzoate (0.1, 0.2),<sup>4</sup> androstenedione<sup>1</sup> (0.4, 0.5), testosterone benzoate<sup>2</sup> (0.53, 0.57), testosterone oxime<sup>1</sup> (0.6, 0.7), testosterone<sup>1</sup> (1.0, 1.0), oreton<sup>2</sup> (testosterone and palmitic acid) (1.4, 1.5), Testosterone diacetate<sup>1</sup> (4.9, 3.3), testosterone acetate<sup>3</sup> (6.3, 4.4), and testosterone propionate<sup>1</sup> (6.6, 4.5). When a comparison of the amount of material necessary to produce the same effect as 0.24 mgm. of testosterone per day is made the values for the last three compounds become diacetate (5.6, 4.4), acetate (10.1, 9.6) and propionate (10.1, 9.6).

Urine extracts prepared by the method of Kochakian and Murlin<sup>5</sup> have an activity equivalent to 1.0, 1.4<sup>4</sup> mgm. of testosterone per liter of urine.

The efficacy of testosterone is markedly diminished as the injections are

<sup>1</sup> Synthetic crystalline material and

<sup>2</sup> Sesame oil solutions in ampules which were diluted with the stock olive oil as required, were provided through the courtesy of Doctors Stragnell and Schwenk of the Schering Corp.

<sup>3</sup> Ampules containing 5 mgm. per cubic centimeter in sesame oil were provided through the courtesy of Doctor Haskell of Ciba Company.

<sup>4</sup> First figures represent seminal vesicles and second figures prostate.

<sup>5</sup> Kochakian, C. D. and J. R. Murlin. *J. Nutrition* 10: 437, 1935.

changed from daily doses to every other day. This is true not only of short term experiments (6 days) but also for prolonged experiments (19 days). On the other hand the propionate, acetate and diacetate will give very nearly similar results when the same total dose is administered in 6 daily doses or in 3 doses on alternate days.

*The effect of androgens on the nitrogen content of the seminal vesicles and prostate of castrate white rats.* CHARLES D. KOCHAKIAN (introduced by J. R. Murlin). Department Vital Economics, University of Rochester, Rochester, N. Y. (Read by title.)

In previous reports<sup>1,2</sup> it has been shown that castrate dogs injected with androgens will retain nitrogen as noted by a decrease in the urea fraction of the urinary nitrogen. The nitrogen retained was attributed to the regeneration of the accessory sex organs of the castrate dogs. To confirm to this hypothesis, approximately 60 sets of seminal vesicles and prostates of white rats of varying degrees of regeneration brought about by injections of testosterone and other androgens were analysed for nitrogen.

The nitrogen content of the seminal vesicles increased in direct proportion to the degree of regeneration and had a per cent value of 3.5. The prostates on the other hand had a nitrogen content of 3.5 per cent at 30 mgm. weight and this value decreased with increase in weight of the organ until at 130 grams weight it was 2.7 per cent at which it remained constant.

*An electromagnetic recording flowmeter.* A. KOLIN (introduced by L. N. Katz). Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, Ill. (Demonstration.)

The method is based upon the induction of an electromotive force in a conducting liquid flowing so as to cut magnetic lines of force at right angles (Proc. Soc. Exper. Biol. **35**: 53, 1936). In the case of blood velocity determinations, this potential difference can be tapped without injury to the blood vessel by placing two non-polarizable electrodes in contact with the vessel wall. The blood vessel is snugly fitted into a bakelite sleeve of smaller internal diameter, and containing two channels drilled through at right angles to the axis of the vessel. The sleeve is placed between the two poles of an electromagnet so that the channels are at right angles to the magnetic lines of force. Saline soaked wicks are inserted in the channels and connect the blood vessel by means of two non-polarizable boot electrodes to the input of an AC amplifying system.

A 50 cycle tuning fork interrupter in its input circuit enables one to record constant or fluctuating flows. A potentiometer in series with the blood vessel permits compensation of undesired potential differences.

The voltage generated in the flowing blood is linearly proportional to the rate of flow; hence the calibration curve can be obtained by a single flow measurement. The rate of flow at each instant is recorded on a moving film without significant distortion by a suitable oscillograph in the amplifier output. The shape of the recorded curve is a true picture of the velocity changes. The area beneath the recorded velocity curve is proportional to the amount of liquid which passes through a cross section of the vessel. The mean flow can be derived readily from this area measurement. Calibration without opening vessel is possible.

<sup>1</sup> Kochakian, C. D. and J. R. Murlin. J. Nutrition **10**: 437, 1935.

<sup>2</sup> Kochakian, C. D. and J. R. Murlin. Am. J. Physiol. **117**: 642, 1936.

This method has been used to follow changes of flow through the carotid artery of anaesthetized dogs and to determine the shape of the carotid velocity pulse under various conditions. Flow pulsations were also recorded from a carotid artery loop enclosed by skin.

*An analysis of the afferent and efferent motor mechanism in the frog by longitudinal and transverse sections at various levels of the encephalon.* BEATRICE G. KONHEIM (introduced by F. H. Pike). Columbia University, New York City. (Moving pictures.)

About seventy frogs have been used. After the cut has been made in the brain of a normal frog the following observations have been made:—Behavior in water, manner of floating, righting reflex, ability to jump from water, swimming technic; Behavior on ground, jumping technic, degree of extension, speed of flexion, unnatural swinging or thrust of legs, evidence of loss of tactile or muscle sense indicated by odd positions of limbs, method of landing from spring, presence of spontaneous movement; Response to visual stimuli; Response to vestibular stimulation by tilting, linear acceleration, and rotation experiments. The observations were made as soon as the frog recovered from the effects of the anesthesia and continued until the frog showed signs of weakness or until no further changes were noticed. It was desired to compare the results with those seen in mammals.

*Results.* Diencephalic longitudinal split. Effect on movement slight. Some loss of spontaneity in movement. Changes direction frequently in jumping so that little distance is covered.

Diencephalic transverse section at extreme caudal border—bilateral. No response to visual stimuli. No normal response to vestibular stimulation. Keeps head lowered when disturbed. Some spontaneous movement shown but slow and indecisive.

Diencephalic transverse section at extreme rostral border—bilateral. Movements slower, less spontaneous and animal quieter.

Mesencephalic longitudinal split. No noticeable effect on movement.

Cerebellar extirpation. Vestibular responses normal. Tendency to exhibit hyperextension of legs with a rigid position of arms and wide flinging of legs. When landing from jump body hits ground heavily instead of spring, with feet partially extended. Hyperextension may increase until movements become convulsive in character.

Rhombencephalic longitudinal split in rostral half of medulla. Some or all vestibular response lost depending on extent of cut. Righting reflexes poor particularly in water, legs hyperextended and flung sideways in jumping. In some cases arms overextend lateralward, swung too high in "walking" or buckle under body as if some sensory disturbance were present.

*The influence of theelin and the gonad stimulating principle of the anterior pituitary on calcium metabolism in rachitic dogs.* F. L. KOZELKA and H. J. TATUM (introduced by A. L. Tatum). Department of Pharmacology, University of Wisconsin, Madison.

It was found that 13.3 Steenbock rat units of vitamin D were sufficient to maintain a normal calcium level in mature thyroparathyroidectomized female dogs, but during oestrus, 26 or more units were necessary to prevent tetany. Much of the evidence on the effect of internal secretions asso-

ciated with the reproductive organs on calcium metabolism is conflicting, since certain investigators observed that the sex hormones induce marked augmentation of the serum calcium while others found a fall in calcium or no appreciable change.

Rachitic dogs, in which 20 units of parathyroid hormone per kilogram of body weight raised the calcium level 0.9 mgm. or less, were treated daily with 37.5 rat units of the gonad stimulating principle of the anterior pituitary or 1,000 rat units of theelin, for a period of four days. The serum calcium decreased in both lots. The average calcium level in the animals treated with anterior pituitary extract dropped from 10.1 to 8.4 mgm. per cent, and in those treated with theelin from 10.6 to 9.1 mgm. per cent. The subsequent administration of 20 units of parathyroid hormone per kilogram of body weight caused the serum calcium to rise to its previous level.

*Action potentials from the heart of the early chick embryo.* M. R. KRASNO (by invitation), J. A. E. EYSTER and J. P. HETTWER (by invitation). Department of Physiology, Wisconsin University Medical School, Madison.

Potential time curves were recorded from points in a circular conducting field surrounding the primitive cardiac tube of the early chick embryo. These curves show that the disposition of electrical charges responsible for the action potential is of the same type as in the adult vertebrate heart. This disposition, characteristic of heart muscle, is present as soon as a spontaneous rhythm is established (40-45 hrs. of incubation) and before the primitive cardiac tube has acquired the syncytial structure typical of adult heart muscle. The organ at this stage is a simple tube of nearly uniform diameter.

*Abnormalities of the startle pattern.* CARNEY LANDIS (introduced by H. B. Williams). Department of Psychology, New York State Psychiatric Institute, New York City.

Within one-half second after the sound of a pistol shot a clear, unmistakable, immediate, stable, reflex pattern of response may be regularly obtained in monkeys, apes, infants, children and normal adult human beings. This "startle pattern" is best demonstrated by ultra-rapid cinematography (64 to 2400 exposures per second). The main features of the pattern are shutting the eyes, a characteristic distortion of the features, forward movement of the head, raising and drawing forward of the shoulders, abduction of the upper arms, bending of the elbows, pronation of the forearms, clasping of the hands, forward movement of the trunk, contraction of the abdomen, and bending of the knees. The pattern varies in degree of manifestation among individuals and in any one individual from time to time but its general outline is uniform for all normal subjects.

Extension of this investigation to psychopathological and neurological patients brought out the following facts. 1. The pattern is not altered in the manic-depressive psychosis, involutional melancholia, alcoholism or general senility. 2. Many catatonic schizophrenic patients show a gross exaggeration of the pattern. 3. The pattern is usually diminished in general paresis. 4. One-fourth of epileptics fail to show any element of the pattern. This absence of response is not related to medication, severity

of disease, physical or mental deterioration, or any other available neurological or physiological finding.

*"The startle pattern". "The startle pattern in psychopathological patients".*

CARNEY LANDIS and W. A. HUNT (introduced by H. B. Williams). New York State Psychiatric Institute, New York City, and Connecticut College, New London. (Motion picture.)

Two twelve-minute motion picture presentations showing the nature of the immediate pattern of response to the sound of a pistol shot. The first of these films uses ultra-rapid motion picture photography with camera speeds of 64, 500 and 1600 exposures per second. This film shows certain of the conditions which change or govern the appearance of this pattern of response. The second film, taken at 64 exposures per second, demonstrates the disorders of this pattern of response in various psychopathological groups such as dementia praecox, involutional melancholia, manic-depressive, epilepsy, amentia, etc.

*After discharge in the central nervous system in response to electrical stimulation of the cerebral cortex.* M. G. LARRABEE and J. P. HENDRIX (introduced by D. W. Bronk). University of Pennsylvania, Philadelphia.

It has been shown (Adrian: *J. Physiol.* **88**: 127, 1936) that repetitive electric shocks applied to the cerebral cortex of the anesthetized rabbit, cat, or monkey are capable of initiating activity in cortical neurones which outlasts the period of stimulation. The electrical sign of this activity was recorded through electrodes placed on the surface of the ipsilateral cerebral cortex.

We have produced this type of after-discharge by stimulating the suprasylvian gyrus of the cat's cerebral cortex for several seconds with strong electric shocks at a frequency of twenty or more per second.

This self-maintained activity has several interesting features: 1. The after discharge spreads widely through the central nervous system. It can be recorded from the contralateral as well as the ipsilateral cerebral cortex and from the cerebellar cortex. 2. The activity sometimes continues for more than two minutes after the end of stimulation. 3. The discharge is often organized into very simple patterns, as also observed by Adrian.

When recorded through concentric electrodes three millimeters in outside diameter placed on the surface of the cortex one to three centimeters from the stimulating electrodes the after discharge consists most typically of regularly spaced sharp spikes at frequencies of twenty to two per second. The frequency usually decreases and the heights of the spikes increase toward the end of the activity. This part of the response is frequently preceded by a period of a disorganized activity from which the spike pattern gradually emerges. Occasionally the discharge is disorganized throughout. Frequently there are abrupt changes in its form.

The duration of the after-discharge frequently increases with the duration of the preceding stimulation. As strength or frequency of stimulation is increased the duration of stimulation required to produce an after-discharge decreases.

Two periods of stimulation, each of such short duration that it can not of itself elicit an after-discharge, may summate to produce effective excitation even when separated by more than four seconds.

*The influence of the inferior mesenteric ganglion on the mechanical excitability of the dog's colon.* HAMPDEN LAWSON and A. M. LEIGH, JR. (by invitation). Department of Physiology and Pharmacology, University of Louisville School of Medicine, Louisville, Ky.

In lightly barbitalized dogs the large intestine was divided into segments about five centimeters long by threading safety-pins transversely through the gut wall. A balloon approximately 10 cm. long was inserted into each segment through an incision in the antimesenteric border. Inflation of the balloon produced an increase in length as well as in the transverse diameter of the segment. Responses obtained were similar to those described for Trendelenburg loops of the large intestine. Responses were usually more constant and of larger amplitude than when the segments were delimited by complete ligation, probably because the latter method blocks all nerve fibres in the gut wall, especially those of the sacral outflow.

Segments in the caudal half of the colon responded to distention typically with an initial period of relaxation during which all contractions were suppressed, lasting from one to five minutes. This was followed by a period of increased secondary tonus waves, often with a general tone rise, the peak of the excitatory response usually being reached within ten to fifteen minutes.

After division of the spinal rami of the inferior mesenteric ganglion there was usually more complete suppression of contractions during the period of relaxation, tone during this period often dropping to lower levels than before. The frequency of the secondary tonus waves appearing during the period of excitation was reduced, and the height of maximal contractions was increased.

After subsequent division of the hypogastric and lumbar colonic nerves the secondary tonus waves appearing during the excitatory period were still further slowed. In slightly more than one-half the animals the height of maximal contractions was increased significantly. During the long intervals between the tonus waves, tone frequently dropped lower than in the ramisected controls. Initial relaxation responses were in some cases converted into initial excitatory responses.

The data offer further evidence for automaticity in the decentralized ganglion, and suggest as a primary function an acceleration of the secondary tonus rhythm of the caudal segments of the colon.

*Further evidence for the indirect control of skeletal muscle tone and fatigue by the autonomic nervous system.* C. E. LEESE and A. FOGELBERG (by invitation). Department of Physiology, The George Washington University School of Medicine, Washington, D. C.

The authors have shown that there occurs, with the use of bulbocapnine in several species, a peripheral circulatory change which is associated with a decrease in skeletal muscle tone and rapid onset of fatigue. Studies made 1, upon the discharge of the central nervous system into the muscle; 2, upon optimal rates of stimulation to maintain a "steady state"; 3, upon arterial and venous blood pressure changes, and 4, upon cardiac activity, minimize their importance and show that the abnormal skeletal muscle behavior in bulbocapnine catatonias depends largely upon the mechanisms controlling peripheral vascularity.

Capillary studies upon skeletal muscle, skin, stomach, intestine, heart and liver suggests a depression of the central vasomotor mechanism with bulbocapnine.

The vascular response to adrenin, acetyl-beta-methyl-choline chloride, pitressin and histamine suggest a decreased irritability of the peripheral vasomotor mechanism in animals intoxicated with bulbocapnine.

The depression of the vasomotor system with bulbocapnine decreases the ability of the skeletal muscle to respond with either a constant or varied tone to meet the general changes occurring within the integrated animal.

Parathyroidectomy studies show that the occurrence of tetany is neither checked or enhanced by the peripheral changes associated with bulbocapnine. The fundamental factors underlying tetany seem to be unaltered when the autonomic nervous system is disturbed by the above procedure.

*A new method for the quantitative assay of "follicle-stimulating" substances.*

LOUIS LEVIN and H. H. TYNDALE (introduced by P. E. Smith). Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

Graded doses of C. U. (urinary gonadotropic material of ovariectomized and post-menopausal women) have been injected into groups of 21-23 day old female albino mice. A study of ovarian, uterine and vaginal responses indicates that the increase in uterine weight is the most sensitive indicator of the amount of C.U. administered. A typical preparation, in doses of 0.09, 0.12, 0.16, 0.20, 0.24 and 0.36 mgm. produced uteri (free of intra-uterine fluid) averaging 9.9, 14.6, 20.2, 24.3, 36.4 and 44.7 mgm. respectively and representing increases of 68, 147, 242, 312, 517 and 658 per cent over the control weight. This and similar data leads to the conclusion that by means of uterine weight determination, C.U. potency can be assayed with an accuracy of at least 25 to 30 per cent. This method therefore permits quantitative determinations at least four times as accurate as are possible by means of study of ovarian weight increase.

Since uterine weight increases are elicited by quantities of C.U. but one-tenth to one-fifth as great as those required to produce comparable ovarian reactions, the uterine method is especially advantageous when toxicity or lack of material prohibits the administration of large quantities.

Assays are carried out by dividing the total dose into three equal portions, injected at 24 hour intervals with autopsy 72 hours after the first injection. At this time the weights of the uteri and of the ovaries are considerably greater than at 96 hours after the first injection.

One mouse unit has been tentatively defined as the smallest amount of material, administered in the above manner to each of five or more mice, which produces 100 per cent increase in the average uterine weight.

*Autoneutralization of acid in the stomach.* W. B. LEWIS and GEO. SLAGLE (introduced by W. R. Bloor). From the Biochemical Laboratory and the Gastro-enterological Clinic of Battle Creek Sanitarium, Battle Creek, Mich. (Demonstration.)

Two hundred cubic centimeters of 0.5 per cent HCl are run into the stomach of a man and a dog. Every  $\frac{1}{4}$  hour later fractional analysis is made on both. There is a marked fall in acidity, but the amount of  $\text{Cl}_2$  is hardly changed. This indicates that there is no dilution of acid in the stomach, but a partial neutralization of it by some alkali. Further analyses demonstrate presence of all pancreatic ferments (trypsin, lipase, etc.) in the stomach towards the end of the experiment. Conclusion: the alkali neutralizing acidity in the stomach belongs to the pancreatic juice.

*Further analysis of the conditioned reflex method in relation to the experimental neurosis.* H. S. LIDDELL, GEORGE F. SUTHERLAND (by invitation), RICHARD PARMENTER (by invitation), QUIN F. CURTIS (by invitation) and O. D. ANDERSON (by invitation). Department of Physiology, Cornell University Medical College, Ithaca, N. Y.

Our experiments have proceeded upon the hypothesis that the experimental neurosis so frequently develops in the course of conditioned reflex experiments because the conditioned animal cannot, through procrastination or evasion, avoid making difficult decisions. Systematic variations in the amount of neuromuscular freedom permitted the sheep and pig during conditioning to food and shock have been related to the nature and predictability of the animal's conditioned responses as well as to features of behavior indicative of "tension states" before the onset of the experimental neurosis. As motor outlets (such as are employed in locomotion, opening of the food box, etc.) are blocked signs of nervous tension appear while the conditioned responses become stereotyped and predictable.

*Gastric motility of a fish during hunger and digestion.* EARL R. LOEW (by invitation) and T. L. PATTERSON. Departments of Physiology, Wayne University College of Medicine, Detroit, Mich., and Hopkins Marine Station, Stanford University, Pacific Grove, Calif.

Comparative studies of the gastric motility of various vertebrate and invertebrate animals have been extended to include the bullhead or cabezone (*Scorpaenichthys marmoratus*).

All the fish were stomostomized and kept in a vivarium containing fresh, aerated, sea water while the gastric motility was recorded by the balloon-manometer method. The manometric pressure varied as the fish changed its depth in the water and the locomotor activity was also recorded. Records of digestive and hunger activity were obtained from six fish.

During periods of fasting (72 to 479 hours) the stomach exhibited rhythmical contractions at an average rate of approximately one per minute. The contractions varied in intensity usually ranging from 50 to 70 mm. of bromoform. The active contractions were completed in twenty seconds and were separated by a period of quiescence lasting forty seconds. No tonal variations were recorded.

Food material remained in the stomach until the third to fifth day of fast. The rate and duration of the rhythmical contractions and the duration of the quiescent intervals were nearly identical during digestion and prolonged fasting. The intensity of the contractions was greater during digestion, the force often being equal to 100 to 160 mm. of bromoform.

The gastric activity during hunger and digestion was continuous, although in several cases during hunger the rate and amplitude of the contractions were diminished for two to three hours. These alterations in activity could not be correlated with any external influence, and may possibly be similar to the alterations or modified periodicity exhibited by the turtle.

The gastric motility exhibited during hunger or digestion was inhibited when small quantities of sea water or weak acid and alkali were introduced directly into the stomach.

The general characteristics of the gastric activity of the fish are similar in nature to those exhibited by the turtle and the bullfrog.

*The central control of postural reactions in the lizard.* WILLIAM P. LONGMIRE, JR. (introduced by Philip Bard). Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

The method of ablating portions of the brain has been used to analyze the central management of postural reactions shown by lizards. *Cnemidophorus sexlineatus* was used in fifteen acute, *Crotaphytus collaris* in twenty-eight chronic experiments. The chronic preparations were operated on aseptically and observed for some weeks. The limits of the ablations were determined histologically.

Posture remained essentially normal after removal of the telencephalon and anterior portion of the mesencephalon. Transections immediately anterior to the red nuclei, however, produced hypertonicity in the most frequently used antigravity muscles of the neck, back and forelegs, yielding, as respects these muscles, a posture resembling decerebrate rigidity. The hindlegs, which are normally less frequently employed by this animal in antigravity functions, were not involved. Unilateral destruction of the red nucleus caused disappearance of the rigidity contralaterally; bilateral destruction produced a flaccid preparation, which showed slight temporary extension of the extremities only during progression. Animals with more caudal ablations involving the anterior portion of Deiter's nuclei developed at the onset of progression a hyperextension of all four legs. This increased with continuance of locomotion (cf. Bagley and Langworthy, 1926).

The placing and hopping reactions seen in higher forms were represented by a single stereotyped response elicitable only on proprioceptive stimulation, the central control of which apparently lies in the posterior medulla. Transections through the anterior portion of Deiter's nuclei seriously impaired but did not completely abolish this response. More caudal transections of the medulla resulted in death of the animals. However, the placing-hopping response of the hindlegs was abolished by transection of the cord at a mid-dorsal level, indicating that the cord alone cannot mediate the reaction.

Labyrinthectomy greatly impaired righting but did not completely abolish it. Poor visual and body righting reactions were demonstrable. Righting was little affected by suprabulbar ablations, but was abolished when the removals extended far enough caudad to involve Deiter's nuclei.

The results will be considered from the point of view of comparative neurophysiology.

*On the mechanism of death in cells killed by high pressures.* B. J. LUYET. St. Louis University, St. Louis, Mo.

The death curves of yeast cells subjected to pressures from 2000 to 6500 atmospheres were established 1, in terms of the intensity of the pressure; 2, in terms of its duration; 3, when the pressure was exerted in hypertonic solutions; 4, when it was exerted in hypotonic solutions; 5, after the cells were injured by heating; 6, after they had been frozen, and 7, after they had been treated by various toxic agents. The lethal pressures are higher in hypotonic, and lower in hypertonic solutions; quantitative relations are discussed. The injuries produced by the different factors are, in general, additive, though a reversal of the order in which they are applied may reverse the effect. The time factor indicates a relatively long and gradual inactivation of the living molecules.

*The effect of ether anesthesia upon the plasma volume of dogs.*<sup>1</sup> F. F. McALLISTER (introduced by M. I. Gregersen). University of Maryland School of Medicine, Baltimore.

In 14 experiments on 9 dogs, etherization for 1 to 2 hours reduced the plasma volume (measured with the blue dye, T-1824, and spectrophotometric analysis of serum samples) between 8.3 and 17.2 per cent, the average being 11.9 per cent. The decrease estimated from the plasma protein concentration (refractometer) ranged from 7.0 to 12.9 per cent, the average being 9.0 per cent. The increase in hematocrit was greater than could be accounted for by the fall in plasma volume. As a rule, these changes occurred within 15 to 30 minutes. Prolongation of the anesthesia to 2 hours did not effect any further decrease in plasma volume unless the depth of anesthesia was also increased. Within 2 to 3 hours after the removal of ether, the plasma volume either returned to normal or rose above it.

*On the nature and distribution of factors for facilitation and extinction in the central nervous system.* WARREN S. McCULLOCH. Laboratory of Neurophysiology, Yale University, School of Medicine, New Haven, Conn.

Comparison of the motor responses with the electrical alterations of cerebral cortex and cord induced by repeated electrical stimulation of the motor cortex show two factors for facilitation and two for extinction.

There is negative afterpotential with decreased threshold (facilitating), followed by positive afterpotential with increased threshold (extinguishing), both maximal at site of stimulation. There is electrical afterdischarge, hyperactivity in reverberating chains (facilitating), followed by deficiency of spontaneous action potentials, hypoactivity (extinguishing), both demonstrable in structures functionally related to the focus stimulated.

By appropriate choice of the physical characteristics of the stimulus, the spacial and temporal distribution of these factors may be made so dissimilar that their part in determining the size of response is distinguishable.

With stimulation designed to avoid motor afterdischarge, local threshold changes associated with D. C. potentials predominate at the site; but even during extinction there, facilitation with electrical afterdischarge appears at neighboring motor foci.

With subthreshold stimulation designed to produce motor afterdischarge at threshold, prolonged electrical afterdischarge appears not only throughout that subdivision of the sensori-motor cortex (except area 6) but also in the ventral white and gray of the corresponding enlargement of the cord, and is associated with facilitation. Subsequently cortical action potentials are practically gone in the areas involved and extinction is demonstrable.

The local rise in threshold associated with positive afterpotential is dependent only on the actual firing of the nerve cells there, and, as afterdischarge signifies prolonged, frequent firing of nerve cells wherever it occurs, similar threshold changes with positive after potential supervene,

<sup>1</sup> Aided by a grant (to Dr. M. I. Gregersen) from The Rockefeller Foundation.

which may account for the loss of spontaneous activity. Thus the factors themselves may be interrelated.

The spacial separation is not absolute. All four factors can be demonstrated electrically at the site of stimulation. By setting up subthreshold background stimulation of five pulses per second and, through the same electrodes, superimposing short periods of stimulation of high pattern and pulse frequency, it is possible to demonstrate the influence of all four factors, and arrive at some conclusions as to their significance.

*Vitamin B-deficient diets and insulin tolerance in the albino rat.* A. R. McINTYRE and J. C. BURKE (by invitation). The University of Nebraska Medical College, Omaha.

Rats upon a normal diet withstood daily intramuscular injections of 200 units of insulin per kilogram body weight for 5 days without discernible effects. The same rats placed upon a diet of untreated casein, cornstarch, lard and the Osborne-Mendel salt mixture supplemented with cod liver oil (thus containing no B<sub>1</sub> and only very small amounts of the remaining B-complexes) were rendered comatose and in many instances killed by daily injections of only 20 units per kgm. after developing weight losses of 8 to 15 per cent. This was only 1/10th the amount of insulin they had previously tolerated. This enhanced effect of insulin cannot be explained entirely by malnutrition as control animals deprived of all food showed no marked sensitivity to insulin in 20 unit per kgm. daily doses until they had lost 25 per cent or more in body weight. Rats allowed to develop weight-losses of 17 to 20 per cent on a vitamin-B<sub>1</sub> free diet supplemented with ample supplies of vitamin B<sub>2</sub> complex from autoclaved yeast (pH 6-7 and heated to 120°C. for 3 hours) were found to tolerate easily five daily doses of 20 units of insulin per kgm. These rats showed only slight depression following the last two doses and in no case was there a rat rendered comatose or killed. In this preliminary report we come to the conclusion that the albino rat on a B<sub>1</sub>-free diet supplemented with autoclaved yeast shows a higher tolerance for insulin than a rat on a diet low in all the vitamin-B complexes.

*The tolerance of the albino rat for insulin.* A. R. McINTYRE and J. C. BURKE (by invitation). University of Nebraska Medical College, Omaha. (Read by title.)

In the course of other experiments it was observed that the albino rat is normally highly resistant to insulin. Rats when allowed free access to food will tolerate 500 units of insulin per kilogram injected intramuscularly during a period of 3 days without showing any discernible effects. (J. S. Latta, personal communication.) In this laboratory rats have been found to tolerate daily injections of 200 units per kgm. without the appearance of symptoms. On the basis of the common conception of the utilization of 2 grams of carbohydrate per unit of insulin the rat will tolerate daily sufficient insulin to utilize 40 per cent of its entire weight expressed as carbohydrate. Rats completely starved until approximately 20 per cent of the body weight was lost were still able to tolerate a total of 100 units of insulin per kilogram injected over a five day period, or sufficient insulin to utilize more than the entire weight of the animals expressed as carbohydrate.

*The rate of growth of albino rats with and without daily injections of insulin.*

A. R. MCINTYRE and J. C. BURKE (by invitation). University of Nebraska Medical College, Omaha. (Read by title.)

Recently there has been presented clinical evidence which appears to indicate that insulin may cause weight increments in man. This investigation was made in order to ascertain what effects the daily administration of insulin has upon the weight-gain curve of normal rats of both sexes when fed an adequate diet. Twenty-two rats (litter-mate pairs) from 125 to 200 grams in weight were divided into two groups and the rate of growth of each animal was determined by daily weighings through a 14 day preliminary period. One group was then given daily injections of 20 units of insulin per kilogram (the albino rat is highly tolerant to insulin) for a 21 day period. The growth rate being again determined by daily weighings. There was no difference in growth-rate in either group either before or after the insulin injections. It is concluded that insulin in these doses administered to rats upon an adequate diet for the above duration of time has no effect whatsoever upon rate of growth.

*Action potentials from the gastrocnemius muscle of the frog.* C. A. MAASKE (by invitation), M. R. KRASNO (by invitation) and J. A. E. EYSTER. Department of Physiology, University of Wisconsin Medical School, Madison.

Previous work from this laboratory has shown that the potential-time curves recorded from a circular conducting field around the frog's gastrocnemius muscle result from two coaxial dipoles, oriented along the long axis of the muscle. The present work considers in more detail the position of the dipoles in the muscle, their approximate length and whether they are to be regarded as stationary or in motion. Action potentials were recorded from contacts 1 mm. in diameter, and spaced 1.6 mm. apart, along the long axis of the muscle. These contacts led through non-polarizable electrodes to the grid of a two stage amplifier. The muscle, mounted under nearly isometric conditions, was completely immersed in a bath of Ringer's solution contained in a circular dish. The circuit was completed by an electrode at the edge of the conducting field at right angles to the long axis of the muscle, leading to the ground input of the amplifier. Action potential curves were thus made from 18 to 20 points along the surface of the muscle from its proximal to its distal end. A second string galvanometer recorded the constant action potential from two fixed points near each end of the muscle.

By plotting the two maxima of the action potentials against distance along the muscle, it was found that the potential distribution in the muscle corresponds to two effective dipoles. The distance between positive and negative charges (length of dipole) is approximately 7 to 9 mm. while the proximal charges lie about six millimeters from the femoral end of the muscle. These two "effective" stationary dipoles may result from two sets of dipoles moving in opposite directions from a certain point in the muscle. Investigations in this connection are being continued.

*The absorption of fats, oils and other chemicals through the skin.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Powerful drugs and poisons incorporated in ointments or lotions of fixed oils and fats, applied to the intact skin of animals, were found to penetrate the surface but poorly although lanolin was better absorbed than the other fats. The essential oils, similarly tested, contrasted strikingly with the fixed fats. When applied to the skin of mice, rats, guinea pigs and rabbits, these oils, most of which are official flavoring agents, were readily absorbed, as indicated by pharmacological and chemical reactions. Pure chemical constituents found in volatile or essential oils—phenols, ethers, esters, ketones, terpenes, etc.—were tested separately and were found to be readily absorbed through the skin. The author similarly employed some of these aromatic compounds as vehicles to convey into the body various other medicinal agents. In toxicological experiments with nicotine alkaloid a great difference in penetrability was noted between normal and pathological skin areas. While nicotine applied to normal shaven skin of rats and guinea pigs was readily absorbed and produced toxic symptoms, the penetration of the alkaloid was greatly retarded by prior freezing, scalding and chemical injuries of the skin of the animals. In all such experiments, of course, the animals were kept and finally dispatched under ether anesthesia. Both the pharmacological and toxicological findings obtained in this research should prove to be useful guides in rational pharmacotherapy and dermatological practice.

*Physiological comparison of tri-brom ethanol and some homologues.* DAVID I. MACHT and R. V. RICE (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The chemically pure compounds, tri-methyl ethanol, tri-ethyl ethanol, di-ethyl-mono-methyl ethanol and di-methyl-mono-ethyl ethanol, were prepared and compared with ethyl alcohol and with tri-brom ethanol (avertin, Winthrop Co.) in respect to their physiological properties. Experiments with 10 per cent solutions in alcohol and one per cent solutions in olive oil were made on mice, rats, guinea pigs, goldfish, tadpoles, cats, isolated smooth muscle, muscle oxydase and *Lupinus albus* seedlings. Mice, intraperitoneally injected with 0.75 cc. of avertin in olive oil, 1 per cent, exhibited a deep narcosis lasting for ten hours or more and sometimes terminating fatally. Tri-ethyl ethanol was the most narcotic of this series of four compounds, 1 cc. of a one per cent solution producing a narcosis of two hours' duration. Tri-methyl ethanol was the weakest of the series and the other members were narcotic in proportion to the number of ethyl groups present in the molecules. Toxicity of the compounds for goldfish, tadpoles and *Lupinus* seedlings was also proportionate to the number of the ethyl group in the homologues. Avertin, however, was more toxic than any of the series. In toxicological experiments with rats in a maze, 10 mgm. of tri-ethyl ethanol were much more depressant than 10 mgm. of tri-brom ethanol. Cats under ether, injected intravenously with 50 mgm. of the respective compounds in 9.5 per cent alcohol, exhibited transient depression of the respiration and blood pressure. The most toxic compound in these experiments was found to be di-methyl-mono-ethyl ethanol, which paralyzed the respiration and stopped the heart. Smooth muscle, in general, was depressed by all the compounds. The activity of muscle oxydase was also inhibited in varying degrees by the different compounds, the tri-brom and tri-ethyl ethanol being the most depressant.

*Phytotoxic reactions of dry blood.* DAVID I. MACHT and HILAH F. BRYAN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

When examining the toxicity of blood by plant-physiological methods, the senior author for many years has been employing small quantities of serum because whole blood and plasma obtained with the aid of such anticoagulants as oxalates, citrates and fluorides are harmful for plant protoplasm. Whole blood or plasma can be employed for phytopharmacological work, however, if it is kept fluid with highly purified heparin. While normal blood serum, one per cent, gives a phytotoxic index of 70 to 75 per cent, whole blood gives a reading of 65 to 70 per cent. A method for detecting toxicity of dry blood films was devised as follows: Freshly drawn blood is kept fluid with highly purified heparin (H. W. & D.). A clean microscope slide is spread with 0.25 cc. of such blood, which is allowed to dry in a thin film at room temperature. Whenever desired, two such films, equivalent to 0.5 cc. of whole blood, are taken from the ice-box and dissolved in 50 cc. of plant-physiological solution. The resultant solution is then tested for its toxicity for *Lupinus albus* seedlings by methods which the senior author has described in numerous prior publications. Thus it was found by comparison of the solutions of the dissolved films with fresh blood specimens taken at the same time the dry films were made that the former, obtained from both normal and pathological cases, retained their toxicity even after having been kept for several months. Specimens of normal and of menstrual blood and sera from cases of pernicious anemia, respectively studied in this manner, were found to retain their toxicity. It was learned, furthermore, that the toxicity of blood preserved in dry films was much more efficiently retained than that of fluid blood which had been frozen solid and kept in cold storage. The procedure described above promises to be of considerable value in diagnostic and medicolegal work.

*Effect of cobra venom on paralysis agitans.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

In connection with a research on the mechanism of analgesia produced in higher animals by cobra venom, the author studied the effect of the drug on convulsions of cerebral origin, elicited pharmacologically. It was found that cobra venom antagonized the convulsant effect of subsequently injected camphorated oil and of santonin, drugs which are generally regarded as affecting the higher nervous centers. The central action of cobra venom in relieving pain and quieting convulsions suggested its use in patients with Parkinson's disease, or paralysis agitans, who complained of excruciating pain. Six cases have so far been studied. In three of these, there was not only relief of pain effected but also definite amelioration of spasticity and tremor. In three other such cases, not suffering pain, the cobra venom had a beneficial action on tremor and spasticity. Further controlled clinical studies on this subject are in progress.

*Physiological action of salicylal-amino pyridine and brom-salicylal-amino pyridine.* DAVID I. MACHT and H. A. B. DUNNING, JR. Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

These compounds were synthesized and sodium salts were prepared in

concentrations of 1:200 to 1:100 for pharmacological examination. The most remarkable effect produced by the compounds was their action on the central nervous system. Four milligrams of salicylal-amino pyridine, injected into a mouse, produced violent clonic and tonic convulsions, and



death in three minutes. The brominated compound, however, was relatively less convulsant. The same quantity injected into a mouse produced milder convulsions from which the animal recovered. When injected in rats, the two chemicals were similarly convulsant, the brominated compound again proving the less active. When both compounds were tested on surviving animal tissues (smooth muscle) and plant tissues (*Lupinus albus* seedlings), the brominated one was much more toxic than the other. Intravenously injected in cats under ether, these compounds markedly stimulated respiration and the vasomotor center and effected a sharp rise in blood pressure, which did not fall for a long time. The absolute lethal dose of the brominated compound (75 mgm. per kilo) for cats was somewhat smaller than that of the unbrominated one (100 mgm. per kilo). The latter markedly stimulated the frequency and force of contractions of smooth muscle, while the brominated compound was less stimulating. Comparison of the two chemicals indicated definitely that the bromine atom incorporated in the organic molecule is responsible for some sedation but increases the toxicity of the compounds in other respects.

*Physiological effect of extract from cicadas.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Periodical cicadas, or seventeen-year-old locusts (*Magicicada septendecim*), were gathered in Baltimore, dried at room temperature and divided into male and female groups. The wings of the insects were then clipped off and the abdominal portion of each grasshopper was separated from the anterior part of the body. A batch of material was extracted with hot alcohol, 70 per cent, in a reflux container. Chloroform extracts were similarly prepared for physiological experimentation. Alcohol and chloroform were respectively driven off on the water-bath and the residue was taken up in physiological saline. Mice and rats injected with such extracts exhibited toxic symptoms. Intravenously injected in cats under ether, the respective locust extracts depressed both blood pressure and respiration. *Lupinus albus* seedlings, grown in one per cent solution of the extracts, were markedly inhibited. The abdominal portions of the locusts contained the greatest amount of toxic substance and extracts obtained from the male cicadas were much more active than those derived from the female in all the physiological and pharmacological experiments.

*Physiological studies of grasshopper extracts.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Large quantities of grasshoppers (*Melanoplus differentialis*) were extracted with cold 90 per cent alcohol. After the alcohol had been evaporated and the residue dissolved in physiological saline, the physiological effects of the extracts were studied. Injected in mice, they produced marked poisoning. Blood pressure and respiration of cats under ether anesthesia were much depressed by intravenous injection of the extracts, the most potent of which was that made from the posterior or abdominal portions of the male insects. Injections of saline extracts markedly depressed the neuromuscular system of white rats, as indicated by their behavior in a circular maze. *Lupinus albus* seedlings, grown in one per cent solutions, were greatly inhibited, the extracts from the abdominal portion of the male insects being much more toxic than those obtained from the posterior section of the female grasshoppers. The most interesting finding was the discovery of vitamin content of alcoholic extracts of grasshoppers. Examination of the extracts by nutrition experts revealed that they contain considerable quantities of vitamin A and are also rich in vitamin B-1. Further studies with regard to these and other vitamins are in progress.

*Comparison of indole-acetic, indole-butyric and alpha-naphthalene-acetic acids on plants and animals.* DAVID I. MACHT and MARY L. GRUMBEN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

These compounds, which have been studied by Zimmerman, Wilcoxon and Hitchcock and described as plant-growth-promoting drugs, were tested on *Lupinus albus* seedlings, on the one hand, and on mice and cats, on the other. One milligram of the two indole derivatives, injected intraperitoneally in mice, produced excitation, tremor and convulsions. Half a cubic centimeter of the alpha-naphthalene-acetic acid produced convulsions and death within a few minutes. Five milligrams of each, intravenously injected in cats under ether anesthesia, produced transient arrest of the respiration and a marked fall in blood pressure, the weakest compound in this respect being the indole-acetic acid and the strongest alpha-naphthalene-acetic acid. *Lupinus albus* seedlings were markedly inhibited in root growth after exposure for half an hour or longer in solutions of 1:1,000,000,000 of the respective chemicals. Definite stimulation of root growth was noted, however, after immersion of the seedlings in solutions of 1:1,000,000,000 for shorter periods of time or in still weaker solutions for longer periods. The study revealed that the order of toxicity of the three acetic compounds for mice and cats is the same as that noted by the plant physiologists in their botanical experiments; indole-acetic acid being the weakest, indole-butyric more potent and alpha-naphthalene-acetic acid being the most poisonous of the series. Of greater interest, however, was the fact that living plants were affected by all three of these so-called "plant hormones" in much weaker doses than those required to poison animals.

*Physiological action of some di-benzyl ethanol amides.* DAVID I. MACHT and KENNETH BRIGHTON. Laboratory of Organic Chemistry, Johns Hopkins University, and Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Pharmacological studies were made of the following compounds,  $(\text{C}_6\text{H}_5\cdot\text{CH}_2)_2\cdot\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{OH}\cdot\text{HCl}$ ,  $(\text{C}_6\text{H}_5\cdot\text{CH}_2)_2\cdot\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\cdot\text{CH}_3$ ,

and  $(\text{C}_6\text{H}_5\cdot\text{CH}_2)_2\cdot\text{N}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{O}\cdot\overset{\text{O}}{\underset{\text{O}}{\text{C}}}\cdot\text{C}_6\text{H}_5$ , prepared under the direc-

tion of Professor E. Emmet Reid. When these compounds were tested for their local anesthetic action, it was found that while the hydrochloride and the benzoic ester produced some local anesthesia, the acetyl ester did not. The anesthesia produced by the hydrochloride, the most potent compound, was but one-tenth of that effected by cocaine. Intraperitoneal injection in mice of the acetyl compound and the hydrochloride, respectively, produced convulsions, coma and death. The benzoic ester was much less toxic. The acetyl compound was the most toxic for goldfish placed in concentrations of 1:5,000, but all the drugs stopped their respiration. Similar effects were produced on tadpoles. In tests on *Lupinus albus* seedlings grown in 1:5,000 solutions, the acetyl compound exerted the most toxicity and the hydrochloride the least. When the respective compounds were intravenously injected in cats under ether anesthesia, the hydrochloride and acetyl derivative produced stimulation of the respiration and rise in blood pressure; the benzoyl ester had very little effect.

*Changes in action potentials of the central mechanism controlling breathing produced by modifications of the respiratory act.* CONWAY MAGEE (by invitation), JOHN BRICKER (by invitation) and ROBERT GESELL. University of Michigan, Ann Arbor.

Modifications of respiration in dogs decerebrated under morphine-urethane or evipal anesthesia were produced by artificial ventilation, expiratory and inspiratory mechanical asphyxias,  $\text{NaCN}$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{CO}_2$ . Potentials were obtained by means of needle electrodes from the dorsal funiculus, gracile and cuneate nuclei, internal arcuate fibers, medial and lateral reticular formations, reticulo-spinal tracts, ventral horn cells, and connections to and from the cerebellum.

On the whole, there was a similarity of response to these procedures whether the structure was on the sensory, motor, or internuncial portion of the arc, i.e., inspiratory or expiratory potentials were either augmented or diminished; inspiratory and expiratory potentials occurring with each respiratory cycle were altered in a parallel or reciprocal manner.

Most structures showed respiratory potentials in only one phase of breathing. Identical structures might on one occasion show inspiratory potentials and on another, expiratory potentials.

In some experiments expiratory and inspiratory potentials were recorded from the same location. When both occurred during normal breathing a procedure might selectively eliminate potentials from one phase. When potentials occurred in only one phase they might be changed over to potentials of the opposite phase or potentials of the opposite phase might be added temporarily without alteration of the initial group. Some procedures changed regular, discrete potentials to a rhythm conforming to respiration which suggests that many regularly firing cells may be drawn into respiratory activity. Some stimulating procedures elicited respiratory

potentials from previously inactive regions showing recruitment of intensity and extensity. At times some procedures [administration of  $\text{Na}_2\text{CO}_3$  or  $\text{NaCN}$  (late effect)] increased or created continuous, indiscrete background potentials to such an extent that concurrent respiratory rhythm was either masked or abolished. Occasionally these background potentials were elicited from previously silent regions. Other procedures [administration of  $\text{CO}_2$  or  $\text{NaCN}$  (early effect)] reduced background potentials and accentuated the respiratory rhythm.

These and other results indicate a varied system of potentials which drives the respiratory mechanism. The difficulty of analysis arises from the uncertainty of the exact source of the action potentials—whether they arise from the sensory or motor side of the internuncial connections.

*Alveolar  $\text{CO}_2$ , a physiological variant.* ROLLAND J. MAIN. Department of Physiology, Medical College of Virginia, Richmond.

Some factors producing variations in alveolar  $\text{CO}_2$  of man at rest are: hunger contractions, increased bladder tension, drowsiness, and posture. The effect of these will be illustrated by slides. Stimulation of respiration by hunger contractions, a phenomenon originally discovered by Carlson, will be illustrated by simultaneous records of respiration and gastric contractions.

These variations demonstrate that alveolar  $\text{CO}_2$  tension is not a physiological constant; rather is it a resultant of all factors affecting the respiratory center.

*pH variation in gastrocnemius muscle of intact rabbit after contraction.*

G. L. MAISON and O. S. ORTH (introduced by J. A. E. Eyster). Department of Physiology, Wisconsin University Medical School, Madison.

Present knowledge of the pH changes in muscle due to contraction includes the fact that there is an early alkaline tendency, and a terminal acid change (Meyerhof; Lippman; Margaria; Dubuissin). The magnitude and time relations of these changes have not been worked out, and their presence in the muscle with intact circulation has not been demonstrated as previous experiments have been done on excised or minced muscles.

Using a capillary glass electrode (with indwelling calomel half-cell) inserted directly into the bared gastrocnemius muscle of rabbits under sodium amytal anesthesia, we have been able to demonstrate a definite pattern of response of pH. Stimuli are delivered from a condenser to the cut sciatic nerve at a fixed rate of 5 per second for varying periods.

After a base-line has been obtained, observations must be interrupted during the actual stimulation to avoid action current accumulation as the glass electrode measures potential of whatever source as well as hydrogen ion potential. Readings are resumed about 15 seconds after stimulation has ceased.

The normal pattern of response has three phases: a short alkaline phase, an acid phase, and a prolonged period of gradual recovery to previous pH level. With very short stimuli, the first phase alone, occurs. If the period of stimulation is prolonged enough, the alkaline phase is not seen at all in our observations. The extent of the changes is graded with the length of the stimulation, and for periods under 30 seconds (150 stimuli) the entire change is less than 0.05 pH.

Death of the animal results in an immediate and gradual fall of pH. Sodium iodoacetate given before death eliminates this death acidity entirely. In the living, iodoacetate poisoned muscle, contraction produces no acid phase, but a more prolonged, and more marked alkaline rise occurs.

*The distribution of electrolytes in rat tissues.* JEANNE F. MANERY (introduced by A. Baird Hastings). Department of Physiology, The University of Rochester Medical School, Rochester, N. Y., and the Department of Biological Chemistry, Harvard Medical School, Boston, Mass.

The indirect evidence of the extracellular position of muscle chlorides has raised the question as to whether all chlorides in the mammalian body are extracellular, except those of the erythrocytes. Rat tissues were analyzed to test the assumptions: 1, that all tissue chlorides are extracellular; 2, that the extracellular phase is essentially an ultrafiltrate of serum with less than 1 per cent protein and with concentrations of inorganic ions predictable from the ionic concentrations of serum and the Gibbs-Donnan Law. Chloride, water, blood and fat were determined in a total of 16 different tissues of 7 albino rats. Applying the above assumptions to the analyses of chlorides and water, and calculating their concentrations on the basis of fat and blood-free tissue, we have estimated the percentage of water in the tissue cells, and have compared the values with that known to exist in the mammalian erythrocyte (66 per cent by weight). The tissues analyzed have been grouped according to their per cent of intracellular water: 1, brain, testes, heart, spleen, skeletal muscles, and lungs, 70 to 80 per cent; 2, liver, kidney, stomach and intestine, 58 to 70 per cent; 3, tendon, muscle sheath and skin, less than 40 per cent. Values as low as those found in group 3 suggest the presence of few or no cells inaccessible to chloride. This group is characterized by a low-grade cell structure and a high concentration of the connective tissue proteins, collagen and elastin. Muscle tendon and sheath have such a high chloride concentration that by scraping gastrocnemius muscle bundles free from their sheaths, the muscle chloride concentration was considerably lowered.

Assumption may therefore be valid when applied to tissues with a highly specialized cell structure, but does not apply to those containing a relatively large amount of connective tissue. The data suggest that the second assumption should be modified, and that the tissue "extracellular phase" or "chloride space" might be regarded as closely related to the "connective tissue phase" and as containing a considerable amount of protein. Its significance in relation to the sodium and chloride in muscle will be discussed.

*Representation of tactile sensibility in the monkey's cortex as indicated by cortical potentials.* WADE H. MARSHALL<sup>1</sup> (by invitation), CLINTON N. WOOLSEY (by invitation) and PHILIP BARD. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

Discrete tactile stimulation of a restricted cutaneous area produces in the cortex of the anesthetized monkey a well localized surface positive wave. Applying this fact we have mapped the contralateral representation of tactile sensibility over areas 3, 1 and 2. Only the face has shown bilateral representation. Foci are similar in relative position and extent

<sup>1</sup> Fellow of the National Research Council.

to motor points for corresponding parts. Different surfaces of a part are represented separately. Thus the ventral aspect of the leg appears in orderly sequence on the medial cortical surface between genitalia, at sulcus cinguli, and toe V, at hemispherical rim; the dorsal aspect is represented on the dorsal surface between toe I and trunk.

The potentials, conducted by thread electrodes, are recorded by cathode ray oscillograph. Factors essential for recording these waves are limitation of stimulus duration and restriction of area stimulated. A camel's hair brush or von Frey hair in contact with hairs or skin is moved to and fro through a distance of 1 mm. within 5 ms. Stimulus intensity is just above human threshold, frequency 1 to 2 a second.

Although stimulation of a specific peripheral locus elicits positive potentials detectable over several square millimeters of cortex, one or two discrete spots of maximal potential are always found. Cortical areas vary in size with the peripheral areas represented. At the edges of cortical areas the potentials fall to a small fraction of maximal within a millimeter. The maximal waves vary with the state of the cortex. Voltages range up to 500  $\mu$ v., durations from 15 to 80 ms. Latencies, in milliseconds, are approximately 19 for foot, 13 for hand and 8 for face.

Cortical potentials evoked by limited successive stimulations of a given spot in the peripheral area represented are attenuated or obliterated when a non-discrete, fairly diffuse stimulus (e.g., rubbing) is continuously applied to another part of that area.

*Plethysmographic studies with special reference to waves of respiration.*

S. J. MARTIN and F. S. MARCELLUS (by invitation). Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, and the General Engineering Laboratory, General Electric Company, Schenectady, N. Y. (Read by title.)

A new and sensitive apparatus has been developed which can record waves of respiration along with pulse and volume changes in plethysmographic studies of the toes and fingers in man and monkey and of the fore and hind paws in dogs, cats and rabbits; these waves could not be seen in observations on guinea pigs and rats. The technique is simple and is particularly advantageous in that it affords one a permanent record immediately and without recourse to photographic procedures. Calibration of this apparatus in terms of plethysmographic volume is now in progress.

The following results are reported from plethysmographic studies of the hind paws of 15 dogs and 5 cats in which an attempt was made to determine underlying factors responsible for the appearance of respiratory waves. A definite correlation exists between increase in plethysmographic volume of the paw and phases of respiration. No change is noted upon the normal appearance or character of these respiratory waves after clamping the femoral or common iliac veins, sectioning of the femoral and sciatic nerves, transectioning the spinal cord at the twelfth dorsal and first lumbar region and after the interposition of a glass cannula between the proximal and distal cut ends of the common iliac artery. These waves were still visible in a pulseless and recently dead animal under artificial respiration. However, the respiratory waves disappeared promptly upon clamping the femoral or common iliac arteries or when artificial respiration was temporarily stopped; they reappeared in normal character when the clamps were removed or artificial respiration re-instituted. From these

experiments, it appears that the mechanical or hydrostatic factor is partly responsible for the respiratory waves in plethysmographic records of the paw in cats and dogs. Further experiments are now in progress to determine what rôle the nervous factor may play.

*Unusual kidney volume response to epinephrine.* EDWARD C. MASON and PHILIP HITCHCOCK (by invitation). University of Oklahoma, Oklahoma City.

We have observed very marked increase in kidney volume following the injection of epinephrine in several pithed dogs. We have considered the various factors which may be responsible for such an unusual response; including 1, the obstruction of the venous return from the kidney; 2, possible shock-like condition of the animal with an accompanying state of stimulation of the vasomotor system; 3, direct stimulation of the vasodilator nerve endings; 4, manipulation of the kidney, damaging the vaso-constrictors of the kidney, and 5, the possible destruction of a vaso-constriction mechanism low in the cord.

*The effects of thymus removal on development, calcium metabolism and quality of "egg envelope" in chickens.* GEORGE H. MAUGHAN. Department of Physiology, Cornell University Medical College, Ithaca, N. Y. (Read by title.)

The effects of thymus removal in chickens has been the subject of experimental investigation in this laboratory for a number of years. Early operations (first and second weeks after hatching) have been the rule. In addition to this, biopsies to search for missed or regenerated thymus tissue have caused but temporary slowing of growth.

No significant differences have been noted in the rate of growth or the time of maturity.

Onset of experimental rickets occurred at exactly the same time in thymectomized and control chickens and the severity of the disease was not more pronounced in the operated groups. Blood calcium in chickens, regardless of sex or age, was as high in the operated birds as in the normal controls. X-ray photographs of the tibia and femur bones of the mature chickens showed no bone defects.

Egg production began at the same age in both groups. Careful studies of the eggs reveal no differences in egg quality, including thickness, texture and soundness of shell, thickness of shell membranes, and quality and texture of egg white.

It seems from these experiments that removal of thymus from chickens has no effect upon growth, calcium metabolism, or upon the so-called "egg envelope" of the eggs produced after the hens have matured.

*Arbutin diabetes.* FRANCES Y. MICHEL (introduced by Harold E. Himwich). Laboratory of Physiology, Yale University School of Medicine, and the Theobald Smith Laboratory of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

Administration of 5 grams of arbutin in sterile distilled water subcutaneously twice daily to healthy fasting dogs produced a condition similar to that of phlorhizin diabetes. Arbutin diabetes is characterized by a lowered blood sugar, acetone bodies in the urine, and a dextrose to nitrogen ratio of 1.60, the average value of the ratios 1.45, 1.56, 1.50 and 1.92 in

four different dogs. Unlike phlorhizin diabetes, the voluntary intake of water is small and the amount of urine is scanty unless water is administered by stomach tube. Both phlorhizin and arbutin are phenol glucosides. Salicin, an alcohol glucoside, and amygdalin, an aldehyde glucoside, were also injected into three fasting dogs in dosages ranging from four to thirty grams and from two to twelve grams per day respectively. These drugs produced neither glycosuria nor ketonuria.

*The presence and distribution of histamine-like substances in blood.* DAVID MINARD (introduced by A. J. Carlson). Department of Physiology, University of Chicago, Chicago, Ill.

Other investigators have reported the presence of histamine-like substances in blood of various species, rabbit blood having the highest histamine equivalent of the species studied. These investigators stated that for the rabbit the distribution of histamine between red blood cells and plasma was in the ratio of 18:1.

The present studies, in which electro dialysis was used for the quantitative extraction of histamine, have shown that the histamine-equivalent of electro dialysates of rabbit blood agree quantitatively on both the isolated guinea-pig intestine and the cat blood pressure with the values obtained using trichloroacetic acid extracts.

Employment of quantitative platelet volume studies has led to the finding that the ratio of the histamine-equivalent concentration of whole rabbit blood to the histamine-equivalent concentration of a platelet-rich, cell-free suspension is the same as the ratio of their corresponding platelet volumes. Furthermore, two suspensions containing the same concentrations of platelets but different quantities of corpuscular elements have the same histamine-equivalent concentrations. On the basis of eight determinations rabbit platelets appear to contain on the average the equivalent of 450 gamma of histamine per gram. Platelet-free plasma possesses only about 3 per cent of the total histamine-like activity of blood.

Similar studies in the dog reveal that in this species the platelets account for less than 15 per cent of the total histamine-like activity of the blood.

Therefore, it seems likely that the wide variation in the concentration of histamine-like substances in the blood of various species may depend largely on the different histamine-equivalent contents of the blood platelets. These findings also indicate that histamine may be one of the toxic factors in serum and defibrinated blood.

*Histamine-like substances in blood following trauma.* DAVID MINARD (introduced by A. J. Carlson). Department of Physiology, University of Chicago, Chicago, Ill. (Read by title.)

Attempts by other investigators to detect an increase in histamine-like substances in blood following trauma and in experimental shock have been unsuccessful.

In the present experiments the extraction method employed was that of Barsoum and Gaddum (*J. Physiol.* **85**: 1), which eliminates substances other than those of histamine-like character acting upon the isolated guinea-pig intestine. Investigations revealed that blood returning from a traumatized extremity of the dog contains a distinctly greater concentration of histamine-like substances than venous blood from the normal extremity. The average increase in six experiments was 80 per cent.

No appreciable change was observed in the histamine-equivalent concentration of arterial blood and normal venous blood before trauma and after the animal was in profound shock.

Whether the amount of histamine-like substances added to the general circulation by the traumatized tissue is of any significance in the production of shock awaits further investigation.

*A study of the circulatory failure of guanidine intoxication.* A. S. MINOT.  
Vanderbilt University Hospital, Nashville, Tenn.

Dogs which are experimentally poisoned by guanidine develop as one symptom of intoxication a circulatory failure which is peripheral in origin and is largely produced by a loss of plasma fluid and protein through generally damaged capillaries. As a result of this loss there is a concentration of the cellular elements of the blood and a marked reduction in plasma volume and the absolute amount of circulating plasma protein. The blood pressure gradually falls to shock levels. Intensive calcium medication prevents to some extent the loss of plasma and this effect is evidently one phase of the well recognized protective action of calcium in guanidine poisoning. Much more effective than calcium therapy in respect to the loss of plasma volume is medication with atropine or scopolamine. The mechanism of the protective action of these drugs in this respect cannot be explained at present. The observation however seems to fit in with the idea that guanidine may exert toxic effect through increased parasymphathetic activity. Since guanidine intoxication appears to be a common secondary complication in certain clinical conditions the results presented may eventually have a practical application.

*The nitrogen-sparing action of glucose in phlorhizin and pancreatic diabetes.*

I. ARTHUR MIRSKY, JOSEPH D. HEIMAN (by invitation) and S. SWADESH (by invitation). Department of Metabolism and Endocrinology, Institute for Medical Research, Jewish Hospital, Cincinnati, O.

It is generally believed that glucose does not exert a nitrogen-sparing action in the absence of insulin. However, in view of our observation that the intravenous administration of glucose in amounts adequate to cause a deposition of glycogen in the liver results in a cessation of ketone formation ("fat-sparing") even in the absence of insulin, we considered it of interest to study the effect of such treatment on the nitrogen metabolism of normal, phlorhizinized and depancreatized dogs.

The rate of non-protein-nitrogen accumulation in the blood after bilateral nephrectomy was used as a gauge of nitrogen metabolism. The data thus obtained reveals that the rate of nitrogen metabolism is fairly constant and that it is 30 per cent higher in phlorhizinized dogs and 70 per cent higher in depancreatized dogs receiving no insulin than it is in normal dogs.

The intravenous administration of glucose in amounts which will allow for the maintenance of a blood sugar level of from 1200 to 1500 mgm. per cent in the depancreatized dog results in a decrease in the rate of nitrogen metabolism in all animals. A decrease of 53.6 per cent occurs in the normal, 89.1 per cent in the phlorhizinized and 57 per cent in the depancreatized dogs.

Our previous studies on ketosis indicate that the antiketogenic action of glucose is due to an inhibition of fat catabolism in the liver in consequence

of an increase in the amount of glycogen available for utilization by that organ. If, likewise, the nitrogen-sparing action of glucose is due to a suppression of protein catabolism in the liver, then a similar decrease in nitrogen metabolism should occur after hepatectomy. Our preliminary results reveal that the rate of nitrogen metabolism in the eviscerated dog is the same as that of the normal dog receiving large amounts of glucose.

Our studies suggest that the increased protein metabolism of diabetes is due to a decrease in the amount of glycogen available for utilization by the liver. After glucose administration and glycogen retention, a suppression of protein metabolism occurs in the liver.

*The influence of vitamin B<sub>1</sub> on experimental convulsions.* H. MOLITOR and W. L. SAMPSON (by invitation). Merck Institute of Therapeutic Research, Rahway, N. J.

The influence of vitamin B<sub>1</sub> on experimental convulsions was studied on both normal and vitamin B<sub>1</sub> depleted rats. Convulsions were produced by intraperitoneal injection of thujone according to the method of Sampson and Fernandez which consists in determining the response of groups of rats to graded doses of the convulsant drug. The percentage of animals responding depends upon the size of the dose and is constant within  $\pm 12$  per cent when groups of 20 are used. The dose of thujone necessary to produce convulsions in 50 per cent of a group was determined.

Intravenous injections of single doses of 15 milligrams or subcutaneous injection of 80 milligrams of crystalline B<sub>1</sub> daily for 6 days produced in normal rats no consistent decrease in the response to thujone.

The influence of vitamin B<sub>1</sub> deficiency on the incidence of convulsions was determined at regular intervals throughout the depletion period. Changes in heart rate and nystagmus as well as in weight served as criteria for the state of depletion. The experiment was controlled with a group of rats whose food intake of a complete diet was restricted to that of the deficient animals plus a daily addendum of 10 gamma of crystalline B<sub>1</sub>. Nine week old rats were selected for this experiment because our previous experiments have shown that a continuous decrease in heart rate and thujone sensitivity occurs throughout the period from 4 weeks to 8 weeks of age. A dose of thujone was given which produced convulsions in approximately 10 per cent of the animals at the beginning of the experiment. No change in response occurred during the first two weeks. However, during the remainder of the depletion period both groups showed an increase in sensitivity reaching a maximum of 50 per cent convulsing when marked symptoms of deficiency were evidenced. At this point the administration of either crystalline B<sub>1</sub> or of an unrestricted complete diet restored the animals to their original sensitivity within five days.

It appears that malnutrition causes an increased susceptibility to convulsions which is non-specific for vitamin B<sub>1</sub> deficiency.

*Changes in irritability to auditory stimuli on falling asleep.* F. J. MULLIN (introduced by N. Kleitman). Departments of Physiology, University of Texas Medical School, Galveston, and University of Chicago, Chicago, Ill.

Last year we reported on the intensity of auditory stimulus necessary to awaken the human sleeper at various times during the night. We found that the depth of sleep is more closely related to the motility of the

sleeping subject than to the hour of the night. The present studies were carried out on five adults and two children, using a similar method for measuring the changes in irritability, during the act of falling asleep, either for an afternoon nap or on first going to bed at night. The auditory stimulus was delivered through a loud-speaker near the head of the sleeper. The experimenter observed the subject continuously throughout each experiment, which lasted varying lengths of time up to an hour.

The curve obtained for the threshold of irritability is S-shaped in character, rising gradually for the first ten minutes after the onset of sleep and then showing a much steeper rise during the next ten minutes and finally reaching its peak in about half an hour. This increased threshold to auditory stimuli is maintained for another ten or twenty minutes. Following this the return toward the waking level of response is steeper than the rise was. The main part of the descent of the curve occupies about ten minutes time. A similar curve of decreasing irritability to sound on going to sleep has been obtained by us in the case of dogs also. The exact significance of the S-shape in these curves remains to be elucidated.

*The effect of intravenous calcium injections on the heart.* L. H. NAHUM and H. E. HOFF. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

The intravenous injection of 10 per cent calcium chloride solution at the rate of 2 cc. per minute produced the following changes in the electrocardiogram of ten normal unanesthetized rabbits: *a*, after 1 cc., slowing and P-R delay; *b*, after 2 cc., auricular fibrillation, and occasional coupled beats and ventricular extrasystoles; *c*, after 3 cc. of more, cardiac arrest from which the animal recovered following massage of the heart and artificial respiration. Recovery was accompanied by the appearance of ventricular extrasystoles from many foci, followed by a period of auricular fibrillation, and resumption of the normal rhythm. Changes in the ventricular complex, which seem specific to calcium, developed after injection of 1 cc., and became more marked with increasing amounts of calcium. These were: 1, a progressive increase in the amplitude of S; 2, a widening of the QRS interval; 3, disappearance of the S-T interval, and 4, an alteration in the amplitude and contour of the T wave. Extreme myosis and vigorous intestinal movements were noted.

In a second series of ten rabbits 25 to 50 mgm. atropine sulphate were injected intravenously a few minutes before the calcium chloride, to determine whether vagal activity was involved in the slowing, A-V delay, and auricular fibrillation notes in the first series. These arrhythmias were totally suppressed in eight experiments, and in two appeared for a short period only after 3 to 4 cc. were injected. Ventricular extrasystoles were much more frequent, and in two experiments ventricular tachycardia appeared, culminating in one in ventricular fibrillation. The arrest of the heart with larger doses of calcium was not prevented, and the characteristic calcium changes occurred in the ventricular complex. Myosis was produced despite the atropine.

The injection of calcium chloride in a third series of ten rabbits anesthetized by intraperitoneal injection of 60 mgm. per kilo of sodium amytal was followed by no cardiac irregularities of any type, and the only change produced was the typical calcium effect on the ventricular complex.

From these experiments it was concluded that: 1, vagal activity is in

part responsible for the auricular fibrillation and the early slowing and A-V delay caused by intravenous injection of calcium chloride; 2, suppression of vagal activity by atropine favors the appearance of ventricular rhythms; 3, amytal prevents the development of all types of arrhythmia; 4, calcium produces a characteristic ventricular electrocardiogram; 5, calcium syncope is due to arrest of the heart.

*Preparation of vasodilatin-free intestinal secretory hormone.* E. S. NASSET.

Department of Vital Economics, University of Rochester, N. Y.

Whole small intestines from hogs are turned inside out, washed in tap water, and extracted 24 to 48 hours with 3 liters of acid alcohol (3 cc. conc. HCl/L. of 85 per cent ethyl alcohol) per kilogram of intestine. The alcohol is distilled off at reduced pressure and the concentration stopped at one-sixth of the original volume. This aqueous solution is saturated with sodium chloride. The salt precipitate is dried and extracted with glacial acetic acid. Ether (4 volumes) is added to the acetic acid extract to precipitate the active material. Reprecipitation yields an amorphous, water soluble product that, in doses of 0.2 to 0.3 mgm./kgm. intravenously, excites segments of the jejunum to secrete. Results obtained in both acute and chronic experiments on dogs will be presented.

In most batches of this crude hormone there is a small amount of secretin. Vice versa in secretin, prepared according to Ivy, there is usually some intestinal hormone.

*The reticulocyte increase in blood during emotional excitement.* L. B. NICE.

Department of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

The number of reticulocytes, total number of erythrocytes and amount of hemoglobin in the peripheral blood (ear vessels) of rabbits was determined in the normal quiet state during emotional excitement, and in the recovery period.

It was found that there is a decided increase in the reticulocytes during excitement which is out of proportion to the augmentation in the total number of erythrocytes and increase in the amount of hemoglobin.

In a series of 21 determinations with the blood of normal rabbits in the quiet state, there were 176,040 reticulocytes per cubic millimeter of blood; 6,130,000 erythrocytes per cubic millimeter and 13.7 grams of hemoglobin per 100 cc. of blood. During excitement the reticulocytes increased to 276,710 or 57.7 per cent above the normal number; the erythrocytes to 7,018,000 or 14.5 per cent and the hemoglobin to 15.37 grams or 12.2 per cent.

In a second series of 14 determinations on normal rabbits' blood the number of reticulocytes during the quiet state were 149,000 per cubic millimeter of blood; the erythrocytes 6,132,000 per cubic millimeter and the hemoglobin 14.1 grams per 100 cc. of blood. During excitement the reticulocytes increased to 210,390 or 41.2 per cent above the normal number found; the erythrocytes to 7,013,000 or 14.4 per cent and the hemoglobin to 15.3 grams per 100 cc. or 8.5 per cent.

In a series of 10 determinations on the blood of splenectomized rabbits in the quiet state, there were 130,100 reticulocytes per cubic millimeter of blood; 5,478,000 erythrocytes per cubic millimeter and 12.85 grams of hemoglobin per 100 cc. of blood. During excitement the reticulocytes

increased to 192,500 or 47.97 per cent; the total erythrocytes to 5,667,000 or 3.3 per cent and the hemoglobin to 13.1 grams or 2 per cent.

The decided augmentation in the number of reticulocytes in the blood stream of normal and splenectomized rabbits during excitement indicates that these cells are discharged from the bone marrow at a more rapid rate in emotional states.

*Alterations in breathing from local chemical applications to the floor of the fourth ventricle.* HAYDEN C. NICHOLSON and SIDNEY SOBIN (by invitation). University of Michigan, Ann Arbor.

These experiments have been performed upon dogs anesthetized with either morphine and urethane or pentobarbital. The effects of irrigation of the floor of the fourth ventricle with Locke's solution equilibrated with various tensions of carbon dioxide were studied and compared with the effects of intratracheal administration of carbon dioxide. The Locke's solution was at body temperature. Application of carbon dioxide in this way might cause either acceleration or slowing of breathing though slowing was the more usual effect. Such local application of carbon dioxide was more apt to cause slowing than was intratracheal administration of carbon dioxide and much greater degrees of slowing resulted from such application than ever resulted from intratracheal administration. If intratracheal carbon dioxide caused an extreme acceleration local application of carbon dioxide to the floor of the fourth ventricle usually caused a slight acceleration. Lowering the temperature of the Locke's solution a few degrees below body temperature rendered marked slowing less apt to occur. The latent period of these effects was extremely brief.

Placing a drop of a 1 per cent nicotine solution on the floor of the fourth ventricle in the region of the calamus scriptorius caused a transient slowing of respiration. This was usually initiated by an apnea which might last a minute. The slowing then gradually diminished and had usually completely disappeared within ten minutes. If 5 per cent nicotine was used the initial slowing was usually followed in from one to four minutes by prolonged acceleration. This acceleration was not infrequently observed also with 1 per cent nicotine. These effects, both slowing and acceleration, have been observed following denervation of both carotid sinuses and in cases in which there was very little change in blood pressure. During the period of acceleration stimulation of the saphenous nerve seems to be somewhat more effective in accelerating respiration than normally. The latent period of the nicotine effect is practically zero.

*The glutathione content of frog peripheral nerve.* PAUL A. NICOLL (introduced by F. O. Schmitt). Department of Zoology, Washington University, St. Louis, Mo.

The glutathione content of frog sciatic nerve was determined manometrically by the method of Woodward, based on the specific coenzymatic activity of the nerve GSH in the dismutation of methyl glyoxal to lactic acid by glyoxalase. With excess substrate and constant enzyme concentration, the lactic acid formation (determined manometrically) is a function of the GSH concentration.

Because of the small amount of tissue available and the low concentration of GSH in frog nerve, difficulty was encountered in correcting for the blank reaction between substrate and acetone yeast (enzyme source).

This difficulty was minimized by using the average value of 5 to 15 determinations for each GSH concentration to construct the standard blank curve. Preliminary experiments with other frog tissues (brain, heart, kidney, liver) proved the reliability of this procedure. The average level of GSH in frog nerve was found to be 10 to 15 mgm. per cent.

To test the possibility that oxidized GSH functions as an oxidative reserve, the GSH content of nerves asphyxiated (to point of extinction of the action potential) in CO was compared with partner nerves unasphyxiated. No significant increase was found to result from the anoxia. However, this does not exclude the possibility that sulphhydryl compounds may play an important cyclical rôle in nerve metabolism, particularly in connection with the bound sulphur groups in the nerve proteins.

*The influence of pituitary extracts on oxygen consumption.* D. K. O'DONOVAN (introduced by J. B. Collip). Department of Biochemistry, McGill University, Montreal, Canada.

Evidence of a metabolic stimulant in pituitary extracts, which acts independently of the thyroid gland, is presented. This substance is differentiated from the thyrotropic principle, and causes a transient rise in oxygen consumption, soon after injection, up to +40 per cent in the rabbit. The increased oxygen consumption is not associated with a rise in blood sugar.

*The crytograph ink writer.* F. OFFNER (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Chicago, Ill. (Demonstration.)

The Crystograph is a pen writing oscillograph actuated by a piezoelectric Rochelle salt crystal element. The frequency response curve is substantially flat from zero to about 180 c.p.s. The instrument thus has a wide range of utility in biological as well as physical sciences. The accuracy of reproduction of several wave forms at various frequencies, as compared with the cathode ray oscillograph, will be demonstrated.

*The effect of dietary protein on endocrine function and on the blood picture of female rats.* ALINE UNDERHILL ORTEN (by invitation) and ARTHUR H. SMITH. Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.

Female albino rats, 21 days old, were fed a synthetic diet low in protein (3.5 per cent lactalbumin) but adequate in all other respects. Daily vitamin supplements low in protein, Ryzamin B (Burroughs-Wellcome Co.), Liver Extract no. 343, cod liver oil concentrate, and wheat germ oil, were given to the animals individually and in amounts found to be abundant for normal growing and adult rats. Control animals were fed the same diet but containing 18 per cent protein; inanition and calorie-control groups were also studied.

The protein deficient animals grew slightly, at an average rate of only 0.48 gram per day; skeletal growth was also retarded. At 97 days of age, the animals had the appearance of slightly elongated 25 to 35 day-old normal rats. They were not emaciated but rather possessed ample depot fat. The livers appeared fatty, both grossly and microscopically. There was a rather marked hypochromic anemia with a normal erythrocyte count and a color index of 0.7; the blood of the inanition, calorie, and age

controls, however, contained a normal concentration of hemoglobin as well as cells.

The ability of the reproductive organs to respond to gonadotropic stimulation was tested by administering to 92 day-old protein deficient animals a single injection of pregnant mare's serum. The response of the ovaries and reproductive tract was positive and equal to that of 25 day-old normal rats given the same dose; this indicates that the reproductive apparatus was retarded in development because of lack of sufficient gonadotropic stimulation rather than because of injury by the dietary regime. Assays of the pituitaries of the 97 day-old protein deficient animals and of the various control rats were made on immature female mice. The pituitaries of the low-protein rats were approximately equal in weight and in gonadotropic activity to those of 30 day-old normal animals, which are also immature.

The relative significance of calories and protein in these studies has been considered. Thus far, the data indicate that the quantity of dietary protein is the factor of primary importance.

*Fetal movements in rabbits and cats.* D. S. PANKRATZ and MARCEL J. MARQUESS (introduced by W. R. Amberson). Department of Histology and Embryology, College of Medicine, University of Tennessee, Memphis. (Motion picture.)

Ordinary and colored motion pictures (16 mm.) of foetal movements in rabbits and cats will be shown. These movements have been observed at different stages of the last part of gestation. Both spontaneous movements and response to tactile stimulation have been studied and photographed.

*Preoperative dietary management of albino rats for thyroparathyroidectomy.*

MARY C. PATRAS (by invitation), E. A. GALAPEAUX (by invitation) and R. D. TEMPLETON. Departments of Physiology and Medicine, Loyola University School of Medicine, and Department of Physiology, The University of Chicago, Chicago, Ill.

Prior to this study the albino rats used were kept on a stock diet consisting of Fox Chow *ad libitum*, with bread, meat and cabbage once per week. Littermates of the same sex and as near the same weight as practical were selected at the age of 28 days and divided into three groups. For control 33 animals were continued on a Fox Chow diet. Thirty-nine animals received a standard casein diet which contained 3 per cent of a salt mixture (Harris). The third group of 41 animals received a salt deficient diet which was identical with the standard except for the substitution of starch for the salt mixture.

The animals remained on their respective diets for 10 days at the end of which time they were thyroparathyroidectomized and subjected to a temperature of 88 to 92°F. without food for 24 hours. During this period the animals were inspected 3 times. Each time they were classed as either dead, surviving with tetany or surviving without tetany. An animal was considered as being in tetany if during the examination there was a spontaneous stiffening of one limb or more or if such developed after one-half minute of exercise. The result of these inspections revealed that the group of animals on the Fox Chow diet for this duration of time showed

practically the same mortality as, and only 10 per cent less morbidity than the group on the standard diet.

In the group receiving the standard diet there was a mortality of 56 per cent while in the group receiving the salt deficient diet the mortality was found to be 98 per cent—a difference in percentage of 42, which can be accounted for only by the difference in the salt content of the diet before operation.

Thirty-eight per cent of the animals in the group receiving the standard diet and the remaining 2 per cent of those on the salt deficient diet were recorded as being in tetany.

None of the salt deficient group of animals survived without tetany while 5 per cent of those on the standard diet remained symptom free.

*Some observations on the mechanism of Cheyne-Stokes' respiration.* W. D. PAUL, JAMES A. GREENE and A. E. FELLER (introduced by F. M. Smith). Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.

We have found that periodic breathing associated with heart disease can be changed to regular rhythm following the intravenous administration of aminophyllin (theophyllin-ethylene-diamine) in a vast majority of instances. In most of the cases studied it was found that the venous and intrathecal pressures were increased. Harrison (Arch. Int. Med. 53: 782, 1934) reported that removal of cerebro-spinal fluid relieved cardiac dyspnea in some instances and that there was a relationship between the two pressures. It appeared that the pathologic physiology of Cheyne-Stokes' respiration might be better understood by studying the alterations produced by the drug and therefore the effect of the drug on the venous and intrathecal pressures was studied. The intrathecal pressures were obtained in millimeters of water and a modification of Hussey's method (Med. Ann. District of Columbia 5: 232, Aug. 1936) was used for measuring the venous pressures. These observations were made continuously before, during and after administration of the drug.

The intrathecal pressure fell and the maximum drop occurred almost simultaneously with the disappearance of the respiratory arrhythmia. This reduction of intrathecal pressure appeared to be a paradox until it was observed that the venous pressure fell also. Simultaneous measurements of the intrathecal and venous pressures showed a relationship between the changes in the two pressures. The maximum reduction of venous pressure coincided with the maximum drop in intrathecal pressure and with the disappearance of the respiratory arrhythmia.

The results of this study indicate that Cheyne-Stokes' respiration is converted to a regular rhythm by an improvement of the cerebral circulation and it is postulated that improper circulation of blood to the brain is an important factor in the production of periodic breathing.

*Insulin convulsions following stellate ganglionectomy.* R. A. PHILLIPS and S. B. BARKER (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City.

Cats, given 20 units of insulin per kilogram body weight, intravenously, developed typical convulsions and were then recovered with glucose by stomach tube. Two days later the right and left stellate ganglia were excised. Ten days later the insulin was again injected and typical con-

vulsions were seen. These experiments do not confirm the observations of Drabkin and Ravdin (This Journal 118: 174, 1937) on dogs.

*Motor effects of median longitudinal incision of the decussations of the medial lemniscus and corticospinal tracts in the cat.* F. H. PIKE. Columbia University, New York City. (Read by title.)

Certain questions arise in comparing results of excision of the cortical motor area and median longitudinal incision of the decussation of the corticospinal tracts in cats and other animals. Some are: Why do the motor and postural effects of the first so much exceed those of the second? Does the difference depend upon mere preservation of approximately one-fifth of the corticospinal fibers in the direct and homolateral components? If not, why are the results of the first, done months after recovery from the second, as severe as if no previous pyramidal lesion had been made? Does some peculiar process in the cortex justify the designation "motor center?" Are the effects of cortical lesions independent of afferent tracts? Are the effects dependent upon rupture of afferent tracts alone? If so, what afferent tracts must be ruptured to give all symptoms of cortical excision? Can results of severity equal to, or greater than, those of cortical excision be obtained by restricted lesions of the corticospinal tracts and some afferent tracts somewhere other than in the cortex? Will lesions, of some afferent tract and some motor tract other than the corticospinal give results comparable to those of cortical excision?

Median longitudinal incision of the lower portion of the medulla oblongata, presumably including pyramidal and lemniscus decussations, gives rise to results, immediate and remote, closely simulating those of cortical motor area excision alone, but more severe. Locomotion is regained more slowly. The fore and hind limbs can be abducted widely months after the operation. The animal falls to one side more readily when, after stroking the side of the head and neck, with the animal leaning strongly against the hand, the hand is suddenly withdrawn. The fore feet frequently slide out when the animal leans against the hand. Reaction to touch seems unaffected. The animal occasionally turns a back somersault when the fore feet are stroked. This occurs also in animals in which the pyramidal decussation and cerebellum are not injured.

*The excretion of inulin and phenol red by the chicken.* ROBERT F. PITTS.

Department of Physiology, New York University College of Medicine, New York City.

Two hundred and seventeen comparisons of phenol red and inulin clearances have been made in eleven normal, unoperated, unanaesthetized chickens, and fifteen comparisons of phenol red, inulin and glucose in two phlorizinized chickens. The inulin clearance is essentially independent of plasma inulin concentration between 70 to 700 mgm. per cent, and averages 1.78 cc. per kilogram of body weight per minute. The inulin clearance varies to a slight degree with urine flow, increasing slightly as the flow increases from 0.2 to 1.8 cc. per minute. The glucose and inulin clearances in the phlorizinized chicken are identical.

The phenol red clearance is dependent on the plasma level of the dye, varying from about 2 cc. per kgm. per minute, at plasma concentrations of 100 mgm. per cent, to 25 cc. per kgm. per minute, at levels of 1 or 2 mgm. per cent. The phenol red/inulin clearance ratio varies from about 1.0 at the high levels to 12.0 at the low levels.

The binding of phenol red by plasma protein was determined by ultrafiltration and dialysis through collodion membranes. When the excretion of phenol red is analyzed as to fraction filtered (per cent free times inulin clearance) and as to fraction secreted (total excretion minus fraction filtered) it is found that the amount of phenol red secreted increases with increasing plasma concentration up to 15 mgm. per cent. Above 15 mgm. per cent the phenol red secreted remained essentially constant and averages 1.1 mgm. per kgm. per minute. Phlorizin depresses the secretion of phenol red.

*The urea clearance in the chicken.* ROBERT F. PITTS and IRVIN M. KORR (by invitation). Department of Physiology, New York University College of Medicine, New York City. (Read by title.)

The normal chicken excretes a very small fraction of its total nitrogen in the form of urea (less than 5 per cent) and the blood urea forms a similarly small fraction of the N.P.N. Since the bird is adapted to excrete its nitrogen in a form other than urea, it is a matter of interest to examine the way in which its kidney handles urea. Simultaneous urea and inulin clearances have been made in normal, unoperated unanaesthetized chickens at plasma urea concentrations ranging from 1.0 to 250 mgm. per cent. The urea/inulin clearance ratio at high urine flows averages 0.72 and is independent of the plasma level of urea.

It appears, therefore, that in the bird urea is filtered at the glomerulus and reabsorbed in part by the tubule to approximately the same degree as in the mammal.

*The excretion of creatine in the marine teleost.* ROBERT F. PITTS. Department of Physiology, New York University College of Medicine, New York City, and the Tortugas Laboratory of the Carnegie Institution of Washington. (Read by title.)

Creatinine-N may sometimes make up 50 per cent of the total urinary nitrogen of marine teleosts. The urinary concentration may be high while the blood concentration is normally quite low. An examination has been made of the excretion of creatine in the red grouper, *Epinephelus morio*, in comparison with the simultaneous excretion of inulin.

Inulin and creatine were administered by intramuscular injection. Eighteen hours later the bladder was emptied by catheterization, the urinary orifice closed with a pursestring suture and a blood sample was taken from the caudal vein. 90 to 240 minutes later a second blood sample was drawn; the fish was killed by a blow on the head and the abdomen opened and urine removed from the bladder by syringe.

In all, successful clearance comparisons were made in 104 fish; 67 of simultaneous creatine and inulin clearances, 17 of simultaneous creatine, inulin and creatinine clearances, and 10 of simultaneous creatine, inulin and glucose clearances after the administration of phlorizin. The creatine/inulin clearance ratio is always greater than unity, and is depressed by raising the plasma creatine level, averaging 8.0 at 10 mgm. per cent and 2.0 at 200 mgm. per cent plasma creatine. This is also true of the creatinine/inulin clearance ratio. The simultaneous creatine and creatinine clearances are not identical, the substance with the lower plasma level having the higher clearance. The administration of phlorizin depresses all clearances, but the creatine clearance is greatly reduced relative to the

inulin clearance. The glucose and inulin clearances are identical in the phlorizinized fish, within the limits of experimental error.

It is concluded that a considerable portion of the creatine excreted by the normal fish is excreted by tubular secretion, and that the process of tubular excretion is depressed by raising the plasma creatine level and by phlorizin.

*Spread of reflex action in the spinal cat following intravenous injection of sodium cyanide.* E. L. PORTER and R. K. BLAIR (by invitation). Department of Physiology, Medical Branch of the University of Texas, Galveston.

The tenuissimus muscle in the cat is long and slender and innervated by a comparatively few motor fibers. It contracts as a part of the flexion reflex in the spinal cat and will respond to single shocks applied to the posterior tibial nerve. With a long light straw and delicate registration on the drum the reflex contractions of the muscle near the threshold show the activity of individual motor units. The preparation therefore becomes a delicate indicator of reflex irritability within the cord, the disappearance of a motor unit indicating depression and the appearance of a new unit indicating spread of activity within the reflex center. If 0.5 mgm. per kgm. of sodium cyanide is injected rapidly into the jugular vein there appears after a latent period of some 40 seconds the contraction of one to several more motor units than were in activity before the injection. If now the stimulation to the sensory nerve be discontinued, no contractions at all occur, indicating that the phenomenon is to be interpreted as a spread of reflex activity, and not as excitation independent of sensory stimuli.

*Action of fats introduced into the duodenum on the pyloric sphincter and adjacent portions of the gut.* J. P. QUIGLEY and I. MESCHAN (by invitation).

Department of Physiology, Western Reserve University, Cleveland, O.

Employing unanesthetized dogs provided with cannulae into the stomach and the duodenum, simultaneous records have been obtained by the balloon method of the pressure changes within the distal pyloric antrum, the pyloric sphincter, and from the proximal and distal duodenum. With the distension pressure within the balloons equivalent to 2 cm. of water, continuous rhythmical motility obtains in these locations in the fasting dog. Resistance to stretch (i.e., tone) is only moderate in these regions and the behavior of the sphincter is not suggestive of a stopcock action.

The slow introduction of moderate quantities of neutral fat (cream or egg yolk) into the third portion of the duodenum beyond the distal balloon leads to prompt inhibition of motility and resting level (tone) of the antrum and sphincter. The behavior of the proximal and distal duodenum was less marked and more variable, being a decrease in tone and motility or no effect; augmentation was not observed. Ten cc. of cream inhibited the antrum and sphincter completely for 8 minutes and complete recovery occurred only after 20 minutes. The inhibitory effects were obtained repeatedly in undiminished magnitude whenever repetition of fat administration followed recovery from the preceding injection. Regurgitation of fats frequently occurred.

The influence of fats on gastric evacuation may be explained in terms of the findings. Fats in the proximal small intestine inhibit tone and

motility of the stomach; thus discharge of gastric contents is suspended because of diminished propulsive force. The sphincter not only fails to prevent passage of material but in this situation is actually relaxed (offering less resistance to stretch); thus regurgitation of duodenal contents is made possible. Regurgitation would be favored by the observed excess of duodenal pressure over sphincter and antral pressure. Antiperistalsis was not involved in this regurgitation process.

*Electrical detection of ovulation.* J. REBOUL (by invitation), H. B. FRIEDGOOD and H. DAVIS. Department of Physiology, Harvard Medical School, Boston, Mass.

We have confirmed the observation of Burr, Hill and Allen (Proc. Soc. Exper. Biol. and Med. 33: 109, 1935) that ovulation in the rabbit is associated with a characteristic electrical change. The latter is detected by a vacuum-tube potentiometer activating a moving-coil galvanometer. Continuous photographic records have been obtained of the potential differences between vagina and abdominal wall preceding and associated with ovulation.

The electrical phenomena are the same whether the abdomen is intact or whether it has been opened and the ovaries exposed. During half an hour before the first ovulation, the vagina sometimes becomes increasingly negative. In every case, within a few seconds after ovulation the vagina becomes abruptly negative. The potential difference passes through a maximum of 6 to 10 millivolts within thirty seconds and returns gradually to its former level in ten or fifteen minutes. Sometimes the return is slower with oscillations of 1 to 2 millivolts superimposed on the decline. Each follicular rupture is associated with such an abrupt potential change.

In absence of ovulation or movements of the electrodes, we have never seen spontaneous changes of potential greater than 1 millivolt during experiments lasting three or four hours. Muscular contractions cause no deflections with our slow recording system. The ovary can be handled with gloved fingers without producing deflections greater than 1 millivolt, and often none at all. Withdrawal of the contents of a pre-ovulatory follicle by a capillary pipette does not cause any electrical deflection.

A sharp electrical reaction of 1 to 2 millivolts, opposite in sign to the ovulation potential and enduring not more than 30 seconds over all, may be produced by a variety of procedures, for the most part involving contact with and possibly trauma to the peritoneum. They include manipulation of the ovary in some cases, manual rupture of an ovarian follicle, and dropping upon the peritoneum from a pipette either follicular fluid, 10-per-cent KCl, or physiological salt solution. The constant character of this minor electrical potential suggests that it is a physiological response. Its nature is under investigation, but it is clearly distinct from the ovulation potential.

*Resistance to shortening and to the development of tension in muscle.* ALFRED C. REDFIELD, ELIZABETH ROORBACH (by invitation) and FRANK M. HULL (by invitation). Biological laboratories, Harvard University, Cambridge, Mass.

A muscle suddenly allowed to shorten a small distance during continuous stimulation, as in the experiments of Gasser and Hill (Proc. Roy. Soc. London, B, 1924) "contracts" and thus redevelops tension, when further

shortening is restrained by a spring myograph, at a rate proportional to the difference between the final tension to be developed,  $T_{max}$ , and the tension,  $T$  existing at any time. Since the myograph obeys Hook's law, the amount of shortening,  $-\Delta l$ , occurring after the restraint of the myograph is encountered, is given by  $-\Delta l = T/C$ , where  $C$  is the modulus of elasticity of the recording system. Thus:

$$-\frac{dl}{dt} = K(T_{max} - T)$$

$$\frac{dT}{dt} = C \cdot K(T_{max} - T)$$

Since  $T_{max}$  represents the force which balances the internal stress when equilibrium is reached, it may be assumed to represent the force tending to "contract" the muscle while equilibrium is being approached, and  $T$  is the force opposing "contraction" at any time.  $T_{max} - T$  is the resultant force which determines the rate at which equilibrium is approached.  $K$  and  $C \cdot K$  are numbers representing respectively the resistance to shortening and to the development of tension.

In experiments on the sartorius of *Rana pipiens*, using myographs with which  $C$  varied through a tenfold range (and which permitted the muscle to shorten up to 12 per cent of its resting length), it was found that  $K$  is not a constant. The resistance to "contraction" after the restraint of the myograph is encountered is not determined primarily by the distances through which the muscle shortens in unit time. On the other hand,  $K$  varies inversely with  $C$  and  $C \cdot K$  is approximately constant. The resistance to "contraction" is determined primarily by some process, other than the actual shortening of the muscle, which is associated with the development of external tension.

*Paired sartorius muscles. Stretched 2 mm. and released to resting length while being stimulated*

FINAL TENSION	PERCENTAGE OF SHORTENING	C	K	C.K.
grams				
66.2	12.6	154	0.277	42.6
75.0	8.4	262	0.157	41.0
79.1	4.2	550	0.079	43.1
90.6	1.6	1633	0.027	44.5

It is concluded that in the contraction of muscle against an opposing force, some factor other than resistance to shortening as such must be invoked to account for the delay in the attainment of equilibrium.

*The effect of self selection of diet—food (protein, carbohydrates and fats), minerals, and vitamins—on growth, activity and reproduction of rats.* CURT P. RICHTER, L. E. HOLT, JR. (by invitation) and BRUNO BARELARE (by invitation). Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

Rats given a choice of purified casein, olive oil, dextrose, sodium chloride, calcium lactate, dry yeast and cod liver oil gained weight more rapidly

than animals on the standard McCollum diet. Their vaginal smears showed very regular four day cycles and their activity (in revolutions of the drum) was approximately normal. The value of the protein was 23 per cent of the total caloric intake; of the carbohydrate, 56 per cent; of the fat, 21 per cent. The animals could be divided into two groups, those which selected predominantly olive oil (caloric intake; protein 35 per cent, carbohydrate 12 per cent, fat 53 per cent) and those which selected predominantly sugar (caloric intake; protein 17 per cent, carbohydrate 81 per cent, fat 2 per cent). The animals of the two groups showed practically normal activity and sex cycles. Animals given the choice of the above substances without cod liver oil developed a vitamin A deficiency. When given access to cod liver oil they showed a definite cod liver oil appetite, resulting in the disappearance of the deficiency symptoms. When yeast was omitted, rats developed a vitamin B deficiency. Later when given access to yeast, they manifested a definite yeast appetite, resulting in the disappearance of the symptoms of vitamin B deficiency.

The sodium chloride intake was 0.12 gram per day and the calcium lactate intake was 0.17 gram per day, which closely approximates the amount taken by animals on the standard McCollum diet.

An opportunity to select vitamin E was not offered in this experiment. Despite the lack of vitamin E, eight out of eight animals conceived, four of which came to term and delivered, and four of which did not deliver. Of the four which delivered, two ate or mashed their litters and two took such care of their litters that one-third of their offspring survived and reached the adult state.

*Blood sugar and basal metabolism in pigeons following administration of prolactin and cortin.* OSCAR RIDDLE, LOUIS B. DOTTI (by invitation) and GUINEVERE C. SMITH (by invitation). Carnegie Institution, Cold Spring Harbor, N. Y.

Prolactin shown to be practically free from F.S.H. and thyrotropic hormones (and later heated to 60°C. for 5 hours at pH 8.0) in doses of 5 to 30 units daily for 1 to 8 days increased the blood sugar in all of 22 groups (94 pigeons) tested. Four other groups (18 birds) tested under minute dosage (1 and 3 units daily) showed lower and negligible increases (3 and 5 per cent respectively). The 22 groups given higher dosage showed increases of 7 to 33 (average, 20) per cent in samples drawn 18 to 25 hours after last injection. Though extreme glycemia was never produced definite and marked increases were regularly obtained. The groups studied included normal doves and pigeons; hypophysectomized and hypophysectomized-castrates; hypophysectomized with partial adrenalectomy; thyroidectomized; and partially adrenalectomized. The smallest increase (7 per cent) was obtained from 7 birds of the last-named group. Isosoluble pituitary fractions, free of prolactin but probably containing effective amounts of all other A.P. hormones, have little or no effect on the blood sugar of these birds. Thus in pigeons the diabetogenic response is found to be a response to prolactin.

Cortin preparations of Kendall, of Klein and of Zwemer, increased blood sugar in birds of above-named types at 7 hours after injection (average 15 per cent in 22 tests) but such increases were not maintained for as long as 23 hours (average 1 per cent in 12 tests).

B.M.R. determinations (at 25° and 30°C.) done 6 to 15 hours after

cortin usually showed a slight increase (+5 per cent in 57 tests) possibly due to a slower absorption of food in cortin-treated pigeons. Prolactin is again shown to increase the B.M.R. in either completely thyroidectomized or completely hypophysectomized pigeons. The effects of prolactin on both blood sugar and B.M.R. are probably partly a result of the hormone's action on the adrenals.

*The "electrical axis" of the heart.* JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Departments of Pharmacology and Anatomy, College of Medicine, Syracuse University, Syracuse, N. Y.

Studies of the electrical axis have been unsatisfactory because, although it is axiomatic that simultaneous points must be defined, a few investigators have actually done so. In the majority of clinical tracings published by Carter (*The fundamentals of electrocardiographic interpretation*, Thomas, 1937)  $R_2$  does not equal  $R_1$  plus  $R_3$ . In such cases there exists no legitimate method of calculating the R axis.

Recording the three leads synchronously, it is easy to verify Einthoven's equation and to observe that (since in one half of normal students the R peaks are not simultaneous) there exists no one electrical axis to calculate. It follows that axes, at 5 or 10 sigma intervals, should be recorded throughout the cycle of invasion, occupation and retreat.

The  $R_2$  axis in intact dogs exhibits a normal range of  $60^\circ$  to  $80^\circ$ , changing  $\pm 5^\circ$  with posture,  $\pm 20^\circ$  with displacement of the heart within an intact pericardium, and  $\pm 100^\circ$  after the pericardium is resected. In normal medical students, the  $R_2$  axis approximates  $40^\circ$  to  $80^\circ$ , shifting  $\pm 10^\circ$  with posture and  $\pm 50^\circ$  with forced respiration.

The S-T axis should be zero, and if not according to DeWaaert (*Am. Heart J.* 12: 184, 1936), points to the locus of an infarction. The entire interval is affected in thrombosis, and may then be modified by rotation of the heart, but if the myocardium is uninjured only a portion of the interval changes with rotation. Granting that the form of an electrocardiogram can be affected by the position of the electrodes, and by the mediastinal contacts of the heart, these variables are readily controlled. It is observed that the S-T changes characteristic of specific muscle infarctions may be produced without visibility altering the situation of the heart, furthermore this constancy of position is substantiated by intrinsic evidence in the constancy of the  $R_2$  axis at  $70^\circ \pm 5^\circ$  before and after such infarction.

*The conducting system.* JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Laboratories of Pharmacology and Anatomy, College of Medicine, Syracuse University, Syracuse, N. Y. (Demonstration.)

Gross and microscopic three-dimensional evidence is available to show that the conducting system of the mammalian ventricle may be divided, for descriptive purposes, into five regions. Three of these: 1, the main bundle; 2, its right and left branches, and 3, the sub-endocardial network are well known. In the latter three or four fingerlike projections of the left branch descend upon the papillary muscles to emerge at the vertex of the heart and supply the external portions of the two superficial muscles. More recently recognized is 4, the intra-myocardial network, a three dimensional system, with ramifications in progressively peripheral planes between muscle layers. Abramson noted that in the right ventricle the

myocardial network is parallel to the surface but that in the left its fibers are mainly radial. (For bibliography see Abramson *Am. J. Anat.* **70**: 250, 1936.) Since the wall of the right ventricle is relatively thin, the radial connecting fibers between planes are short; in the thick left wall these radial connector fibers are naturally longer and more conspicuous. The radial fibers do not enter the most superficial layer of muscle for which a supply from the vertex is available. The hitherto unemphasized *fifth*-division comprises the *terminal fibers* which arise at an acute angle from the myocardial meshwork to proceed some distance in the direction of the muscle fasciculi ultimately supplied. The tubular lattice work about each fasciculus resembles the distribution of blood capillaries to the same muscle cells. In sections of the myocardium histologists have regularly observed this parallelism of Purkinje and muscle cells.

*Conclusion.* If the Purkinje System, as described, conducts the action current, then radial penetration can occur only in the fourth of these five anatomical divisions. Beyond this fourth division, the action current, entering the terminal Purkinje fibers, will advance in a direction parallel to the muscle cells. The innervation of the external portions of the two superficial muscles appears to be exclusively of this character.

*Ammonolyzed adrenaline conjugates and their pressor action in dogs.*

RICHARD G. ROBERTS and HERMAN J. HORVITZ (introduced by L. B. Nice). Department of Physiological Chemistry, Chicago Medical School, Chicago, Ill.

In a previous paper (*Proc. Soc. Exper. Biol. and Med.* **31**: 998, 1934) it was shown that adrenaline when treated with anhydrous liquid ammonia formed an ammonolyzed derivative. This new product proved to be about fifty per cent more effective than adrenaline in producing a prolongation of elevated blood pressure in dogs. The effect on the intensity factor was hardly noticeable as differing from that of adrenaline.

The purpose of the present work was to attempt to conjugate adrenaline with certain other compounds, principally those that are native to the blood stream, using liquid ammonia as a solvent and dispersing medium. The substances chosen for this were glycine, tyrosine, glutamic acid, urea, lactic acid, dextrose, vitamin C and cholesterol. Some of these ammonolyzed conjugates are much more powerful than ammonolyzed adrenaline alone, and will maintain an elevated blood pressure of ten to fifty per cent of the highest rise for one to three hours. The best of these derivatives were made from glycine, dextrose and cholesterol and the poorest from lactic acid and vitamin C. Vitamin C, however, will prevent the oxidation of adrenaline for weeks even in an open beaker if dispersed in ethylene glycol, but not in water.

The anesthetics used were ether and sodium phenobarbital and the vehicles ethylene glycol, glycerol and water. Ethylene glycol is best as water hydrolyzes the product quickly and glycerol is too viscous. The average dose used for a 12 kgm. dog was 0.25 cc. of 1:1000 adrenaline equivalent for a 1:1 molar conjugate of adrenaline and the substances named. No toxic effects were evident. In general the conjugates are not soluble in ethylene glycol, but are injected as finely dispersed sols. It seems probable that the conjugates are coordination compounds held together by the hydrogen bridges of the ammonia. They should have a therapeutic value as hemostatics and as drugs for the treatment of hay fever and asthma.

*Blood pressure in the new born.* M. ROBINOW (by invitation), R. A. WOODBURY and W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

Direct determination of the blood pressure in the umbilical artery has been made in a series of infants. These readings have been compared with readings taken by the usual clinical method using cuffs of varying widths. The readings have been correlated with the maternal blood pressure, the clinical history of the mother and child.

The effect of clamping the cord, taking the first breath and crying will be shown.

*Studies of blood density in man during paroxysms of fever induced by typhoid vaccine and by malaria.* FRED T. ROGERS. Dallas Medical and Surgical Clinic, Dallas, Texas.

A study of the changes of the density of the blood in adult man, measured by the method of Barbour, preceding, during and after paroxysms of fever induced by malaria and by intravenous injections of typhoid vaccine. Determinations of blood density were made simultaneously on both capillary blood (finger tip) and venous blood (cubital vein). There is a short-lasting sudden increase in density preceding the chill and fever. Following this increase in density, the body temperature begins to rise. The blood density returns to normal even though the body temperature is increasing. The fever may continue for several hours after the blood density has returned to its normal level. These facts indicate that in fever so induced in man, the increased density of the blood is a part of the vasoconstrictor anaphylactoid reaction, and of itself, the increase in blood density is not a primary factor in the fever state.

*Erection in the cat following removal of lumbo-sacral segments.*<sup>1</sup> WALTER S. ROOT and PHILIP BARD. Departments of Physiology, Johns Hopkins Medical School and University of Maryland School of Medicine, Baltimore.

Eckhardt (1863) indicated the important rôle of the pelvic nerves in the process of erection. Subsequently it was established that the fibers concerned are parasympathetic and originate in the sacral segments of the cord. We have observed that male cats deprived of sacral cord and the lower two lumbar segments (lumbo-sacral removal) regularly show complete erections when excited by oestrous females (cf. L. R. Müller, 1901). This result has commonly been obtained shortly after emergence from the anesthetic and repeatedly throughout survival periods of several months. Like normal males these animals show erections only after seizing the female. Since the genital region is anesthetic, such erections cannot be dependent on afferent stimuli from this area.

The capacity of a cat to display erections after lumbo-sacral removal is not altered by removal of the lower lumbar sympathetic chains, but is abolished by complete extirpation of the abdominal chains or by inferior mesenteric ganglionectomy alone. Over postoperative periods of several months these males, which are in a zero condition as regards capacity for erection, show no diminution in sexual aggressiveness. So long as the spinal cord remains intact, absence of the abdominal sympathetic chains

<sup>1</sup> Aided by a grant from the Committee for Research in Problems of Sex, National Research Council.

in no way interferes with the ability to intromit nor does it affect the sensibility of the genital region as judged by responses to local stimulation. On the other hand, lumbo-sacral removal alone, with its attendant sensory and motor loss, permanently renders the animal incapable of intromission.

In none of the preparations was evidence of regeneration of nerve fibers obtained.

We conclude that, after removal of the spinal origin of the pelvic autonomic fibers, erection can be mediated by fibers which reach the penis by way of the inferior mesenteric ganglia. These fibers originate above the sixth lumbar segment and course through the abdominal sympathetic chains.

*Intrauterine respiration in the rabbit and human and its significance in relation to the breathing of amniotic fluid.* MORRIS ROSENFELD (by invitation) and FRANKLIN F. SNYDER. The Johns Hopkins University, Baltimore, Md. (Motion picture.)

A motion picture record was obtained of respiratory movements of rabbit fetuses observed through the transparent uterine wall, following exposure of the uterus beneath the surface of a saline bath. Arrest of fetal respiration induced by anoxemia is illustrated. In the human, the rhythmical excursions of the abdominal wall resulting from the transmitted respiratory movements of the fetus are seen. The relation of these movements to the breathing of amniotic fluid is illustrated by sections of lungs taken from fetuses of rabbits in which India ink had been added to the amniotic fluid. Carbon particles are shown in the alveoli.

*The distribution of anhidrosis following interruption of various sympathetic pathways in man.* GRACE M. ROTH (introduced by F. C. Mann). The Mayo Foundation, Rochester, Minn.

Impairment of sweating was demonstrated by the use of heat and the application of cobaltous chloride to the skin of the body. In a few cases pilocarpine hydrochloride was administered intramuscularly. These tests were carried out on patients following various types of sympathectomy. The types of operation were divided as follows: Bilateral cervicothoracic and lumbar sympathectomy; bilateral resection of the intercostal nerves from the seventh to the eleventh inclusive; posterior infradiaphragmatic bilateral splanchnic resection with bilateral removal of the first lumbar ganglia; bilateral ventral rhizotomy from the fifth thoracic to the second lumbar spinal nerves; extensive bilateral splanchnic resection, partial resection of the celiac ganglion, partial bilateral resection of the suprarenal glands and bilateral removal of the first and second lumbar ganglia.

Anhidrosis occurred in the cutaneous areas corresponding fairly closely to the segmental level at which the sympathetic nerves were distributed in the spinal nerves. The area of skin supplied by the grey rami of each ganglion corresponds in the main with the area of skin supplied by the homologous posterior root fibers.

*The constancy of basal metabolism and pulse rate in relation to body temperature and the menstrual cycle.* BORIS B. RUBENSTEIN. Laboratory of Anatomy and Associated Foundations, Western Reserve University, Cleveland, Ohio.

We have previously shown that the curve of body temperature during

the menstrual cycle reaches its peak during the premenstrual week; and its nadir 8 to 21 days postmenstrually, simultaneously with the appearance of the preovulatory-ovulatory vaginal smears. We now present evidence that the BMR of normal women fluctuates 10 to 20 per cent (while the BMR of males varies only 3 per cent) with an average day-to-day change of  $\pm 5$  per cent. The largest day-to-day changes usually occur during the low temperature phase of the cycle. Our data indicate that the resting pulse rates of women are similarly variable, and correlated with the BMR.

Since BMR normally keeps pace with heat dissipation which is under control of the autonomic nervous system; and since the heart-rate, too, is an expression of the autonomic balance, we suggest that the stability of BMR, and pulse rate is a good index of autonomic stability. We also suggest that the low temperature phase of the menstrual cycle is a period of least homeostasis.

TABLE 1

*The basal metabolic rates of ten women*

Taken daily during an entire menstrual cycle, showing the mean values and the deviations during the preovulatory and premenstrual periods.

SUBJECT NUM- BER	ENTIRE MONTH				PREOVULATIVE PERIOD				PREMENSTRUAL PERIOD			
	Mean, cal./hr.	S.D.*	Range, cal./hr.	A.D.** per cent	Mean, cal./hr.	S.D.	Range, cal./hr.	A.D., per cent	Mean, cal./hr.	S.D.	Range, cal./hr.	A.D., per cent
1	54.6	2.2	7.7	3.1	53.9	2.7	7.7	4.3	54.5	1.3	2.7	1.8
2	59.7	4.1	14.8	6.5	60.5	4.9	12.3	11.9	62.8	2.7	8.7	4.0
3	40.0	2.4	10.4	6.0					40.1	1.2	2.7	2.7
4	51.3	1.9	6.6	4.2	51.1	1.3	3.3	3.5	51.9	1.9	4.3	4.8
5	53.9	2.6	9.7	4.3	51.3	2.4	7.2	4.9	55.7	1.6	4.3	3.4
6	52.6	1.5	5.5	3.0	51.6	2.1	5.5	4.1	52.9	1.1	3.0	2.1
7	49.8	2.8	12.8	5.2	47.5	1.2	3.0	2.7	50.7	1.0	2.4	2.2
8	53.8	2.3	10.0	3.9	53.9	3.0	8.2	6.7	56.3	1.7	4.7	3.4
9	52.8	2.7	11.6	4.7	51.1	1.6	4.4	4.0	56.2	2.7	7.1	7.1
10	54.2	2.3	9.2	4.8	52.9	2.5	6.9	5.2	54.6	1.4	2.7	2.6

\* S.D. is standard deviation.

\*\* A.D. is average deviation.

*Further experiments on the somato-sensory functions of the cerebral cortex in the monkey and chimpanzee.* T. C. RUCH, J. F. FULTON and S. KASDON (by invitation). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Two monkeys (Sooty mangabey) trained to discriminate grades of roughness (emery paper mounted on drums that are rotated to gain food) were subjected to complete unilateral parietal lobectomy. In retention tests both exhibited inability to discriminate any but gross differences in roughness. With further postoperative training a considerable discriminatory ability was regained but the preoperative level of performance was never attained. This function appears to be encephalized but rather widely represented in the cerebral cortex.

In a chimpanzee complete unilateral ablation of the postcentral gyrus and the posterior parietal lobule in one stage induced a severe deficit in the discrimination of both weight and roughness. This extensive cortical

lesion did not however completely abolish either function. In an attempt to localize these functions further, unilateral extirpation of the postcentral gyrus (Brodmann areas 3, 1, 2, and a part of 5) was carried out in two chimpanzees. The first, trained in the discrimination of roughness, exhibited a severe deficit in retention tests but with training duplicated its preoperative performance. In the second chimpanzee a similar result was obtained for the discrimination of weight as well as emery paper, except that no deficiency was demonstrable even in retention tests. These functions are therefore not "focally" represented in the postcentral gyrus of the infrahuman primate cortex. Experiments on the sensory function of the posterior parietal lobule will also be reported.

*Inhibitory action of testosterone on the prostatic changes produced in mice with estrin.* HAROLD P. RUSCH (introduced by W. J. Meek). Department of Physiology, University of Wisconsin Medical School, Madison.

The administration of estrin to male mice, rats, dogs and monkeys will produce pathological changes in the prostate which in some respects are similar to prostatic hypertrophy in man. In brief, an increase in the fibromuscular stroma, a hyperplasia followed by a squamous cell metaplasia of the prostatic epithelium, and a dilatation of the bladder results from estrin given to mice over a long period. Subcutaneous injections of from 10-100 R.U. of estrin (progynon B) in oil were given bi-weekly for periods of either 122 or 137 days. Pathological changes were evident after a period varying from 40 to 70 days at which time bi-weekly injections of testosterone (Oreton, 125-250 gamma) progesterone (250-1250 gamma) or dehydroandrosterone (1250-2500 gamma) were started and continued simultaneously with estrin for the remainder of the experiment. Testosterone in doses of 250 gamma bi-weekly caused a reversal of the metaplasia of the prostatic epithelium toward a normal condition but did not decrease the amount of fibromuscular stroma nor reduce the bladder dilatation. Progesterone and dehydroandrosterone in the doses used were without affect. These results suggest that testosterone may be of value in bringing about a regression of some of the pathological changes present in prostatic hypertrophy of men which recent work indicates may be the result of a relative decrease of the male hormone in relation to the estrogenic hormone.

*Adenosine triphosphate in recovery from muscular contraction.* JACOB SACKS. Laboratory of Pharmacology, University of Michigan, Ann Arbor. (Transfer from Pharmacology.)

It has been shown previously that stimulation of the gastrocnemius muscle in cats at the rate of two twitches per second causes hydrolysis of adenosine triphosphate. The present work was undertaken to study the resynthesis of this compound during the recovery period.

The products formed during contraction are: inosinic acid, ammonia, and two molecules of inorganic phosphate. It was found that the initial reaction in the resynthesis is the conversion of inosinic acid to inosine triphosphate; this compound is then reaminized to adenosine triphosphate. The ammonia which was liberated during the hydrolysis is utilized in the resynthesis.

The resynthesis of adenosine triphosphate takes place very slowly in comparison with many other reactions that are observed during the re-

covery period. No evidence was found that could be interpreted as indicating that the energy for the resynthesis of adenosine triphosphate is furnished by the hydrolysis of phosphocreatine. On the contrary, both substances are resynthesized during the recovery period, though not at the same rate.

*Oxygen consumption and respiratory quotient of unstimulated caffeinized muscle.* GEORGE SASLOW. Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

The average oxygen consumption of muscles in Ringer (10 experiments) was  $32 \text{ mm.}^3/\text{gm. (wet)}/\text{hr.}$  ( $\sigma = \pm 4$ ). The average oxygen consumption of muscles in 0.037–0.042 per cent caffeine-Ringer was  $201 \text{ mm.}^3/\text{gm.}/\text{hr.}$  (range 140–268 in 7 experiments) when the solution was in equilibrium with air, and  $528 \text{ mm.}^3/\text{gm.}/\text{hr.}$  (range 360–723 in 10 experiments) when the solution was in equilibrium with oxygen at atmospheric pressure.

The average R.Q. (10 experiments) of muscles in Ringer's solution was 0.89 ( $\sigma = \pm 0.04$ ), that of muscles in caffeine-Ringer 1.00 ( $\sigma = \pm 0.03$ ) in 15 experiments. The difference between the means is seven times its standard error.

The observations indicate that carbohydrate is the chief material consumed in muscles the metabolism of which has been increased to 4 to 24 times the normal rate by treatment with caffeine.

R. pipiens muscles were studied (at  $23 \pm 0.01^\circ$ ) in differential volumeters by the phosphate method of Dickens and Simer. The weights used varied from 100 to 1147 mgm. (wet). Duplicate experiments showed that an uncertainty of 1 to 2 per cent in the R.Q. was generally attainable.

Lactic acid determinations showed that muscles in 0.04 per cent caffeine-Ringer in equilibrium with nitrogen produce 84 and 87 mgm. per cent lactic acid per hour (the resting lactic acid content being allowed for). On subsequent exposure to oxygen, the accumulated acid is removed at the rates of 24 mgm. percent per hour (over  $\frac{3}{4}$  hours) and 15 mgm. per cent per hour (over 5 hours). Caffeinized muscles in equilibrium with oxygen continuously for 3 or 6 hours show the same lactic acid content as untreated muscles in oxygen for the same periods: 20.3 and 19.6 mgm. per cent in the one and 13.4 and 13.4 mgm. per cent in the other experiment. No lactic acid accumulates in caffeinized muscles in oxygen. The observed oxygen consumption of caffeinized muscles in these experiments was 478 and 352  $\text{mm.}^3/\text{gm.}/\text{hr.}$ , that is 71 and 51 per cent, respectively, of the rates necessary to oxidize 84 and 87 mgm. per cent lactic acid per hour.

*The human spiogram.* LEON J. SAUL and FRANZ ALEXANDER (by invitation). The Institute for Psychoanalysis, Chicago, Ill. (Read by title.)

Respiratory tracings of 265 individuals were examined. Curves were obtained by the standard technique for basal metabolisms, except that at the ends of the tests vital capacities were recorded, thus showing the respiratory level (chest level). Curves on our Institute patients were taken on two successive days and repeated every two or three months. They were studied for the relatively "chronic" phenomena of rate, width of tidal air (TA), respiratory level (ERL), degree and frequency of rounding of the inspiratory and expiratory tips (respiratory pauses); and for the "spasmodic" phenomena of "hooks" (slight reversals) during inspiration and

expiration, breath holdings, and inspiratory and (rarely occurring) expiratory "spikes", i.e., sudden deep inspirations, gasps or sighs. A very rough quantitative estimate of rounding of tips was made by estimating the degree and frequency as 0, 1, 2 or 3 plus, and by utilizing the ratio of the duration of inspiration to expiration. Statistical study yielded no constant correlations between the various features of the spirograms except that spikes were rarely present in curves without hooks.

The main features of a person's spirogram are individual and characteristic, somewhat like his handwriting. They are relatively constant (and recognizable) despite variations in details. Of the Institute for Psychoanalysis series, 21 per cent showed considerable variability but still retained a recognizable characteristic individuality. Experiment showed that imitation of another's spirogram is extremely difficult. Statistical studies have thus far failed to reveal any significant correlations between features of the spirograms and body build or chest shape. Relative to the averages of the entire group, fifteen asthmatics showed slightly higher respiratory levels, faster rates, more irregularity, and conspicuously less rounding, hooks and spikes. Spirograms of groups of men and women, normal, psychoneurotic, psychotic, asthmatic, and with gastric ulcer were compared and show certain characteristics. Efficiencies varied from 8 to 32 liters of oxygen breathed for 1 liter absorbed. Averages for the Institute group, 30 males, 36 females, are:

*Males: Rate: 11.6 TA: 522.0 cc. TA per cent: 15.7 ERL per cent: 82.8 VC: 3593.1 cc. Rounding: 0—43.3 per cent, 1 plus—33.3 per cent, 2 plus—10.0 per cent, 3 plus—13.4 per cent. Expiratory Hooks: 0 and 1—76.7 per cent, 2 and over—23.3 per cent. Spikes: 0 and 1—93.3 per cent, 2 and over—6.7 per cent. Females: Rate: 13.5 TA: 417 cc. TA per cent: 18.0, ERL per cent: 85.6, VC: 2453.4 cc. Rounding: 0—50.0 per cent, 1 plus—27.8 per cent, 2 plus—16.7 per cent, 3 plus—5.5 per cent. Expiratory Hooks: 0 and 1—69.5 per cent, 2 and over—30.5 per cent. Spikes: 0 and 1—86.1 per cent, 2 and over—13.9 per cent.*

*The rôle of dicarboxylic acids in brain oxidations.* LILLIAN SCHOEN (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Chicago, Ill.

The colloidal sol, prepared by solution of rabbit brain in water (Cohen and Gerard, *Am. J. Physiol.*, 1935, and *Am. J. Physiol.*, in press), oxidizes lactate and succinate and offers a favorable material for testing St. Gyorgyi's view that succinate-fumarate-oxalacetate forms an intermediate link in the oxidation of lactate (by cytochrome).

Oxygen consumption of the sol was determined before and after addition of lactate and succinate, singly, together, or in sequence. The respiration increase on adding lactate and succinate together is equal to the sum of the increases when they are added separately, indicating that the two dehydrogenase systems act independently rather than *seriatim*. This is true alike at 37°C. and at 20°C., despite marked differences in absolute rates. Further, a second portion of succinate, added to the sol, after the extra oxidations due to a first portion are ended, is initially oxidized more slowly than was the first addition; and the same is true for lactate added after lactate oxidation. But succinate added after lactate has been oxidized, or lactate added after oxidation of succinate, is attacked exactly as when no previous substrate addition has been made. This again favors an independence rather than interdependence of the two oxidation systems.

Finally, added malonate, which inhibits succinate oxidation, 'does not effect that of lactate. It seems, therefore, that lactate can be oxidized by brain enzymes without the intermediacy of a dicarboxylic link.

ORIGINAL SOLUTION	QO <sub>2</sub> BEFORE TIP	TIPPED IN	QO <sub>2</sub> 10 MIN.	QO <sub>2</sub> 1 HOUR
Brain sol*	16.5			
Brain sol	15.6	Na succ. (to .001M)	44.3 (+29.4)	20.7 (+8.9)
Brain sol	15.8	Na lact. (to .01M)	34.3 (+19.4)	17.5 (+5.7)
			(+48.8)	(+14.6)
Brain sol	15.4	Na succ. + Na lact.	57.4 (+42.5)	26.5 (+14.7)
Brain sol†	20.8		13.7	
Brain sol + Na succ.	20.5	Na succ.	35.2 (+21.5)	
Brain sol + Na succ.	17.8	Na lact.	37.7 (+24.0)	
Brain sol + Na lact.	16.6	Na succ.	41.5 (+27.8)	
Brain sol + Na lact.	19.2	Na lact.	21.6 (+7.9)	

\* Average data from nine complete experiments at 37°C. Values at 20°C. about  $\frac{1}{2}$  as great.

† Average data from five complete experiments at 37°C.

*The effect of heat on blood and lymph flow from the gastrointestinal tract.* C. R. SCHMIDT, J. M. BEAZELL (by invitation) and A. C. Ivy. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Heat was applied to the lumen of isolated segments of the small intestine and colon at temperatures ranging between 52.2°C. and 57.7°C., by means of a water heated hollow rubber applicator. Under these circumstances the rate of flow of blood through the treated segment was increased from 200 to 400 per cent unless the mucosa was injured. This procedure had no measurable effect on the rate of flow of lacteal lymph. However the secretion of succus entericus was markedly augmented.

Temperatures of 52.2°C. were applied to the lumen of the stomach without effecting the rate of flow of lymph from the thoracic duct.

When the temperature was maintained at 52.2°C. burns occurred only when the applicator was of such size that it distended the segment or caused it to contract vigorously. When the temperature was 54°C. or more, burns occurred in 3 out of 4 experiments irrespective of the size of the applicator. The contractile response of the colon rendered it more sensitive than the small intestine to the injury produced by too large an applicator. Injury to the treated segment caused a decrease in blood flow and an increase in lymph flow.

*Optical studies of the ultrastructure of the axis cylinder.* FRANCIS O. SCHMITT, RICHARD S. BEAR (by invitation) and JOHN Z. YOUNG (by invitation). Departments of Zoology, Washington University, St. Louis, and Oxford University, England.

Because of their unusually large diameter (400 to 600 $\mu$ ) the giant axons from the stellar nerves of the squid, *Loligo pealii*, permit application of the method of polarization optics to quantitative investigations of the meaning of the axis-cylinder anisotropy in terms of ultrastructure.

Though the Ambronn immersion technique cannot be employed with fresh axons, it has been found that the results of this method as applied to axons fixed in various ways and shrunk by varying amounts can be employed to extrapolate to a hypothetical form-birefringence curve for the fresh axis cylinder. The factor which accounts for the changes in birefringence in the fixed preparations (at constant refractive index) is the

shrinkage of each relative to the original fresh axis-cylinder volume. Thus it is possible for the first time to estimate the relative importances of form and intrinsic micellar anisotropies: the effects due to the micellar shapes are of greater importance than are those caused by intra-micellar dissymmetries, but both are present. The form birefringence, which is positive and uniaxial with respect to axon length, is *qualitatively* that to be expected of a rodlet mixed body of Wiener, but the small *magnitude* of the form effects (the birefringence in the fresh state is  $1.5 \times 10^{-4}$ , while the protein content is about 8 per cent by volume) indicates that the axis cylinder is far from being an ideal rodlet system. Though the optical method is consequently unable to determine a unique structure for the axis cylinder, the data do limit considerably the possible configurations. From the lack of serious discontinuity in birefringence passing from the microscopically homogeneous fresh axon to the well fibrillated fixed material, it is concluded that histological and optical data are best reconciled by considering that the axis-cylinder birefringence is due to a relatively small proportion (about 0.3 to 0.4 per cent of the total volume) of a well oriented anisotropic component, the remainder of the protein being essentially isotropic in shape or unoriented. The more sensitive optical method is apparently better able to detect the oriented component than is the method of x-ray diffraction.

*An electrical theory of nerve impulse propagation.* OTTO H. SCHMITT (introduced by F. O. Schmitt). Departments of Physics and Zoology, Washington University, St. Louis, Mo.

On the supposition that the capacitance of a critical interface in nerve, and perhaps in other irritable tissues, is a function of the potential difference across that interface, an electrical theory of impulse propagation is proposed which correlates the excitation laws, the velocity of impulse propagation, and the shape of the action potential, in terms of a single set of constants.

The assumption of such a variable interface capacitance, is in keeping with the experimental work of Blinks, on the current-voltage relationships existing at cell interfaces and is not contradicted by Cole's measurements on nerve impedance.

Since a mathematical solution of this system would be difficult and unwieldy, an electrical system has been devised in which each of the active components, assumed by the theory to exist in nerve, is identified with an equivalent electrical element in this system.

The validity of the theory is tested by comparing the behavior of this artificial "nerve" with that of real nerve. Many of the familiar properties of nerve, such as the monophasic and diphasic action potential, the strength-duration curve, accommodation, and injury potential have been demonstrated. If the theory is valid and the electrical model is a suitable equivalent, then certain of the unmeasurable electrical characteristics of nerve can be evaluated in terms of the constants in the electrical "nerve" required to make its performance duplicate that of real nerve. The way would then be open for a study of the mechanisms of the effects of abnormal agents such as drugs, ion imbalances, and the like.

*Mechanical solution of the equations of nerve impulse propagation.* OTTO H. SCHMITT (introduced by F. O. Schmitt). Departments of Physics and Zoology, Washington University, St. Louis, Mo. (Demonstration.)

Since solution of the partial differential equations involved in theories of biological impulse propagation is very laborious, and invariably results in complicated expressions which are difficult of application, especially in the case of functional dependence of the circuit elements upon the state of the system, it was thought worth while, when a new theory was to be tested, to develop an instrument which would solve the equations mechanically.

An electrical network was constructed in which each hypothecated element in the nerve was replaced by an electrical element in the network having mathematically identical properties. Thus for example, a first order chemical reaction is replaced by a combination of resistance and capacitance which obeys the same exponential law, while a functional dependence of capacitance upon potential is achieved with the aid of a vacuum tube and relay.

Because of the identical correspondence of many elements of this electrical system with those of nerve, many of the qualitative properties of nerve may be observed in the model directly, without analysis. For example, the monophasic diphasic action potentials, the demarcation potential, the behavior under conditions of polarization and injury, the phenomenon of accommodation, all of these may be observed directly from the electrical "nerve" as from a real nerve. For convenience of observation, however, the potentials are greatly magnified, and the time factor greatly extended, millivolts becoming volts and milliseconds, seconds.

This instrument will be demonstrated in operation and the more striking electrical nerve phenomena will be shown with the aid of the cathode ray oscillograph.

*Further evidence in support of the alarm reaction theory of adrenal insufficiency.* HANS SELYE. Department of Biochemistry, McGill University, Montreal, Canada.

The great similarity between the symptoms of adrenal insufficiency and those produced by any stimulus which elicits the syndrome characteristic of acute generalized damage of the organism which was described under the name of the "alarm reaction" (Selye, H. *Nature* **138**: 32, 1936) and other experimental results reported in a previous communication (Selye, H. *Science*, in press) lead us to assume that the liberation from the tissues of certain toxic metabolites with histamine-like pharmacological effects are the primary cause both of the alarm reaction and of adrenal insufficiency. The opinion has been expressed that these toxic metabolites cannot be detoxified in the absence of the adrenals. This would also explain why adrenalectomized animals have so little resistance against various damaging stimuli.

This theory received confirmation by more recent experiments on the rat which show that saline extracts prepared from the lymphatic tissues (lymph glands, spleen and thymus) of adrenalectomized animals are more toxic for adrenalectomized rats than similar extracts prepared from the lymph glands of normal animals. The toxicity of the lymph glands following adrenalectomy is especially marked if the animals have performed exhaustive muscular exercise shortly before their lymphatic organs were taken for extraction.

It appears that toxic metabolites formed under the influence of alarming

stimuli (in this case exhaustive muscular exercise) accumulate in the lymphatic organs and that they are not detoxified in the absence of the adrenals. The fact that these metabolites are especially toxic for the adrenalectomized animal seems to confirm our conception that one of the main causes of the adrenal deficiency symptoms is the accumulation of toxic metabolites in the tissues which cannot be detoxified in the absence of the adrenal cortical hormone.

*The renal excretion of creatinine in the chicken.* JAMES A. SHANNON. Department of Physiology, New York University College of Medicine, New York City. (Read by title.)

One hundred ten comparisons of the simultaneous creatinine and inulin clearances have been made in 8 normal, unoperated, unanesthetized chickens and 16 comparisons of creatinine, inulin and glucose in three phlorizinized chickens.

At low plasma levels of creatinine (10 mgm. per cent) the creatinine/inulin clearance ratio ranges from 1.35 to 1.8. As the plasma creatinine level is raised the creatinine clearance is depressed both absolutely and relative to the inulin clearance, so that the ratio has a value of 1.10 at 200 mgm. per cent. This is presented as independent evidence of the tubular secretion of creatinine in the bird. If there is no reabsorption of creatinine, the approximation of the creatinine and inulin clearances at high plasma levels of the former is evidence that the inulin clearance in the bird is at the level of glomerular filtration.

In the phlorizinized chicken the inulin, creatinine and glucose clearances are essentially identical. This circumstance, and the fact that high U/P ratios of creatinine were not present in the above experiments, leads us to believe that there was no creatinine reabsorption in either the normal or phlorizinized bird.

It is concluded that in the bird creatinine is excreted in part by tubular excretion and that, as in other animals, the tubular process is depressed by elevating the plasma level of creatinine. The close approach of these two clearances at high plasma levels of creatinine is evidence that the inulin clearance in the bird is at the level of glomerular filtration.

*The renal excretion of inulin and creatinine and the relation between filtration and reabsorption of water in the anesthetized dog and the pump-lung-kidney.*

JAMES A. SHANNON and FRANK R. WINTON (by invitation). Department of Physiology, New York University College of Medicine, New York, and The Physiological Laboratory, Cambridge, England.

The simultaneous clearances of inulin and creatinine have been observed in the dog's surviving kidney, perfused with defibrinated blood, and in the dog anesthetized with chloralose.

In the pump-lung-kidney, at inulin U/P ratios below 20, these clearances are essentially identical. The mean of the 46 creatinine/inulin clearance ratios in this group was 1.018 with a standard deviation of  $\pm 0.053$  and maximum variations of  $+0.092$  and  $-0.098$ . As the degree of water reabsorption increases there is a separation of the two clearances due to an apparent creatinine reabsorption. This creatinine reabsorption does not seem to be due to simple physical diffusion, the extent of which would be dependent upon the concentration gradient and the time available for

diffusion to take place. It is apparently associated with a large increase in the fraction of water reabsorbed which consistently occurs when the amount of water filtered is diminished by lowering the perfusion pressure.

In the anesthetized dog the mean of the creatinine/inulin clearance ratios in 40 comparisons was 0.986 with a standard deviation of  $\pm 0.023$  and maximum variations of  $+0.067$  and  $-0.046$ . The range of inulin U/P ratios examined was 21 to 147. Contrary to the result obtained in the pump-lung-kidney, in the anesthetized dog the fraction of the filtered water reabsorbed is independent of the absolute amount filtered when the latter is varied by varying blood pressure.

The continued identity of the creatinine and inulin clearances following anesthesia with chloralose indicates that they may be used as measures of glomerular filtration in this preparation with the same confidence as in the normal animal. In the pump-lung-kidney their use for this purpose is certainly invalid at high U/P ratios, and possibly so at low U/P ratios.

*Respiration of the optic nerve of the king-crab, with special reference to the relative activity of fibres and sheath.* HERBERT SHAPIRO (introduced by E. Newton Harvey). Princeton University, Princeton, N. J. (Read by title.)

In the king-crab, *Limulus polyphemus*, the optic nerve is quite uniform, consisting of bundles of non-medullated axones surrounded by a non-nervous sheath, and extends from eye to brain over a distance varying from approximately 8 to 12 cm., with only a few minor branches. A disparity in oxygen uptake by different portions of the whole nerve, with a maximum existing along the middle portion of the nerve, was demonstrated by Guttman (1935). Whether this phenomenon is attributable primarily to the axones, or to the thick sheath which surrounds them, or to both, was investigated during the summers of 1935 and 1936, in male *Limuli*, by dividing the nerve into five portions, dissecting away the axones from the sheath with fine glass needles, and measuring the respiration of the ten resulting pieces with the Gerard-Hartline (1934) micro respirometer at temperatures varying from 18° to 28°C. Twenty such series were run, including experiments upon crabs dark adapted for various periods (0.6 day to 16 days). These determinations, together with a number on whole nerves, confirm the original observations of Guttman (1935) on whole nerves and demonstrate in addition that 1, the respiration of the axone material of *Limulus* optic nerve is not uniform along its entire length, but varies, depending upon the region of the nerve selected for measurement; 2, though the axones and sheath are roughly equivalent in bulk (ratio of fibres to sheath, moist weight, varying from 0.7 to 1.6, average 1.2) the respiration of the axones is considerably higher (4 to 5 times); 3, oxygen uptake by the axones is constant over a period of many hours, whereas the sheaths generally show an increasing metabolism, with time; 4, in general, only the axones show the maximum of activity along the middle (more usually, toward the central side) of the nerve; 5, the unequal respiration persists in nerves from dark adapted animals, with a tendency for the maximum to be shifted toward the central end of the nerve (i.e., away from the neurones, which are at the peripheral end, in the eye); 6, temperature does not alter these relationships. Preliminary experiments on frog nerve have likewise resulted in the finding that the respiration is not uniform along the length of the nerve.

*Effects of changes in environmental conditions on skin temperatures and the dissipation of heat from the body.* CHARLES SHEARD, MARVIN M. D. WILLIAMS (by invitation) and BAYARD T. HORTON (by invitation). Division of Biophysical Research and Division of Medicine, the Mayo Foundation and The Mayo Clinic, Rochester, Minn.

In this report there will be presented experimental data and evidence concerning: 1, the rôle of the extremities in the dissipation of heat from the body; 2, the influence of changes of environmental conditions on the temperatures of the skin in various areas in normal individuals in the basal state; 3, the effects of the ingestion of food and the subsequent regulation of the dissipation of the increased production of heat by the upper and lower extremities of the body, under varied but controlled environmental conditions; 4, the relative functions of the upper and lower extremities respectively in the control of the loss of heat from the body, under various environmental conditions, in the basal state and following the ingestion of food; 5, the significance of the deductions, drawn from these investigations, concerning the function of vasoconstriction and the application of the experimental procedure to the study of normal vasoconstriction and peripheral vascular deficiencies.

*A quantitative study of the oxidation of carbohydrate in normal and diabetic men.* J. M. SHELDON and L. H. NEWBURGH (introduced by H. B. Lewis). University of Michigan Medical School, Ann Arbor.

The study consisted of a continuous four hour measurement by means of a respiration chamber of the amount of carbohydrate oxidized by normal and diabetic men. Standard open circuit indirect calorimetry was employed. Our subjects in every case received a maintenance diet of constant composition and adequate in protein for at least three days prior to the determination of the oxidation of carbohydrate. Both groups ingested varying quantities of carbohydrate in the diet of the preliminary period. They took 0 to 200 grams of glucose at the beginning of the chamber study. On the evening of the last preparatory day, the subjects went to bed in the respiration chamber. The next morning, 12 hours or more post-absorptive, they arose, emptied their bladders, drank 400 cc. of water or an equivalent quantity of liquid containing glucose and returned to bed where they quietly reclined for the next four hours. The carbon dioxide and oxygen determinations were begun when the subject had returned to bed.

Fasting normal subjects oxidized increasing amounts of carbohydrate in response to increasing quantities of carbohydrate in the preparatory diet. They likewise oxidized more carbohydrate during the four hours in response to increasing ingestion of glucose even though the preparation had been the same. When both the carbohydrate of the preparatory and chamber periods were simultaneously increased, the oxidation of carbohydrate was additive. The response of the diabetics was qualitatively similar but quantitatively smaller. The diabetics' ability to oxidize glucose was directly related to the severity of the disease.

*The influence of excess vitamin A in the diet upon the uterus and ovary of the rat.* T. C. SHERWOOD, G. P. BIRGE, O. R. DEPP and H. B. DOTSON (introduced by W. J. Meek). Department of Anatomy and Physiology, University of Kentucky, Lexington.

An excess vitamin A diet produces an effect upon the epithelial cells of

the thyroid gland. The normal oestrus cycle as shown by the vaginal smear technic is disturbed for a period of 35 days.

It is the purpose of the present investigation to show that vitamin A affects the uterus and ovary. The hypophysis should be considered if the oestrus cycle is definitely disturbed.

Both feeding and subcutaneous injections giving similar results.

Gross inspection of the uteri and ovaries indicate some variation from the normal. However, microscopic preparations have been made for the study of any possible changes.

*The respiratory metabolism and lactic acid oxidative quotient of excised cardiac muscle of normal and depancreatized dogs.* E. SHORR, MURIEL MALAM (by invitation) and H. B. RICHARDSON. Department of Medicine, Cornell University Medical College and the New York Hospital, New York City.

In contrast to other tissues of the diabetic animal, the heart stores glycogen readily. The mechanism of this storage in the absence of insulin, is not known.

This paper aims to throw light on the mechanism of glycogen storage in the heart under these conditions, by the study of its resynthesis of lactic acid, *in vitro*. The method of determining lactic oxidative quotients was that employed in previous studies from this laboratory (Am. J. Physiol. 1932, 1936). The respiratory metabolism with and without lactate (m/80) of slices of cardiac muscle from normal and depancreatized dogs was measured in the Warburg apparatus. Lactic acid balances were done, concurrently, by chemical methods. These determinations provided the data for calculating the lactic acid oxidative quotient ( $\frac{\text{lactic acid disappearing}}{\text{lactic acid oxidized}}$ )

Quotients above 1.0 indicate resynthesis. The influence of glucose as a substrate, as well as anaerobic glycolysis, was also studied.

**Results: Lactic acid oxidative quotients:** Diabetic cardiac tissue resynthesized added lactate as efficiently as normal heart, to judge from an average oxidative quotient of 3.8 (range 2.3-6.2) as compared with 2.8 (range 0.9-4.0) for the normal. This contrasts with our previous findings with diabetic skeletal muscle, which loses this capacity (this Journal 1932).

**Respiratory metabolism:** The respiratory quotients of normal cardiac muscle in Ringer-phosphate ranged from 0.80 to 0.89, those of diabetic heart from 0.71 to 0.88 (see Himwich et al., this Journal, 1936). Lactate increased the oxygen consumption of the normal heart 57 per cent, and raised the R.Q. an average of 0.11. It increased the respiration of diabetic heart by 34 per cent, but had no appreciable effect on the R.Q. This suggests non-specific stimulation by lactate, rather than oxidation of lactate itself. Glucose caused small, inconstant rises in oxygen consumption of both normal and diabetic hearts, but, while it elevated the R.Q. of normal heart by 0.14, it did not change the diabetic R.Q. significantly. Considerable anaerobic glycolysis occurred with normal and diabetic hearts, and was increased in both by glucose. This, again, contrasts with diabetic skeletal muscle.

**Conclusion.** The diabetic heart, in contrast with diabetic skeletal muscle, retains the ability to resynthesize lactic acid readily. This mechanism may be associated with its capacity to store glycogen.

*Influence of pituitary extracts on renal activity in normal and adrenalectomized opossums.* H. SILVETTE. Physiological Laboratory, University of Virginia.

A number of observations have been made on the urinary excretion (water, sodium, chlorides and urea) of normal and adrenalectomized opossums under various conditions. Adrenalectomized opossums given 100 cc. of 1 per cent sodium chloride solution by mouth showed a remarkable inability to excrete the ingested salt and water within 24 hours. The concentrations of sodium and chlorides per cc. of urine did not differ significantly from normal; the volume of urine, however, was decreased over 50 per cent and thus the total output of salt was correspondingly diminished. Although urine volume was reduced in such experiments, concentration of urea per cubic centimeter of urine compensatorily rose, and the daily excretion of urea remained normal. This observation indicates that following adrenalectomy in the opossum the kidney functions normally as far as nitrogen excretion is concerned. When post-pituitary liquid (Squibb) or Pitressin (Parke-Davis) was administered to adrenalectomized opossums given 100 cc. of one per cent NaCl solution per day, urinary excretion once more became normal. Water, sodium, chloride and urea excretion was thus similar in normal opossums and in adrenalectomized opossums given post-pituitary liquid. It should be emphasized that the administration of post-pituitary liquid to normal opossums leads to no significant change in renal excretion; initial rate and volume of urine flow are, however, somewhat increased.

That the deficiency of the adrenalectomized opossum's kidney to excrete ingested salt and water is one of *rate* mainly is shown by a series of experiments in which animals were kept in metabolism cages for 3 days. These animals were given 100 cc. of salt solution at the beginning of each day, and the urine collected and analyzed at the end of each 24-hour period. If results of three-day periods are compared, the difference in the amount of water, sodium and chloride excreted by normal and adrenalectomized opossums is not nearly as great as the differences observed in a 24-hour run.

The average daily excretion of urea was notably constant in the different experiments, for the concentration of this substance per cc. of urine varied inversely with the amount of urine excreted. Sodium and chloride concentrations per cc. of urine were also rather constant, and the slight reductions in concentration in adrenalectomized animals were offset by the administration of either post-pituitary liquid or cortico-adrenal extract.

*The blood sugar during asphyxia by illuminating gas.* ERMA SMITH and C. W. KIM (by invitation). Iowa State College, Ames.

Separate groups of rats were exposed to 1.43 per cent gas-air mixture (0.34 per cent CO) for varying time intervals up to that required for death. Blood samples were withdrawn from the venae cavae. The micro-method of Folin and Malmros was used for sugar analysis.

Sixty-eight determinations preceding exposure showed the normal blood sugar level to be  $100.1 \pm 0.05$  mgm. per 100 cc. As the per cent of COHb increased the blood sugar increased. The blood sugar at death from CO asphyxia (85 per cent COHb) was  $166.6 \pm 2.1$  mgm. per 100 cc.

A second set of determinations using blood samples withdrawn at 10 minute intervals subsequent to a single sublethal exposure showed a gradual fall in blood sugar with dissociation of the COHb.

*The estimation of the renal blood flow in man.* HOMER W. SMITH and WILLIAM GOLDRING (by invitation). Departments of Physiology and Medicine, New York University College of Medicine, New York City.

It has been pointed out that the whole blood phenol red clearance in man is so large as to suggest that it approaches closely to the actual renal blood flow (Goldring, Clarke and Smith, *J. Clin. Invest.* **15**: 221, 1936). An extended series of determinations on normal individuals gives an average value for the plasma phenol red clearance of 400 cc. per minute, corrected to 1.73 sq. m. surface area. When allowance is made for the hematocrit (since phenol red is carried to the kidneys only by the plasma) this figure corresponds to 665 cc. of whole blood per minute. Estimates based upon the observed blood flow in unanesthetized rabbits and dogs examined under basal conditions lead to the average value of 750 cc. per minute. Though these estimates cannot be accepted as directly applicable to the human kidney, they indicate that the phenol red clearance may be used as an approximate indicator of renal blood flow in man, at least under circumstances where there is little probability of the specific secretory power of the tubules being impaired.

Observations have been made on man during a variety of conditions. The magnitude of the changes in the phenol red clearance justify the conclusions that the renal blood flow, although normally quite constant, is subject to marked changes under certain conditions. It may be considerably decreased by adrenalin (although the fraction of plasma filtered may be increased) and increased by autonomic responses or during spinal anesthesia.

*Alteration in the regulation of respiration occurring at birth.* FRANKLIN F. SNYDER and MORRIS ROSENFELD (by invitation). Johns Hopkins University, Baltimore, Md.

Within the uterus the respiratory response of the rabbit fetus whether premature or postmature was found in earlier experiments to differ from that of the adult, namely low oxygen depressed fetal respiratory movements at a concentration which stimulated the adult, and excess carbon dioxide had little or no effect in the fetus. In the present experiments the attempt was made to determine the time of transition from the fetal to the adult type of response.

Respiratory tracings were obtained from normal newborn during the first ten days following birth and from premature fetuses delivered by hysterotomy at twenty-eight days or later. Results showed that in full term rabbits immediately following birth respiration was stimulated by anoxemia and excess carbon dioxide, as it is throughout later life. The altered response of the newborn is related to birth rather than to the stage of development.

The first appearance of sensitivity to the stimulant effect of excess carbon dioxide and low oxygen was traced back to premature fetuses removed from the uterus at twenty-nine days, i.e., at the beginning of viability. At an earlier stage regular rhythmical respiration occurred but no response of stimulation to excess carbon dioxide or anoxemia was demonstrable.

*The rôle of calcium in the excitation of nerve.* D. Y. SOLANDT (introduced by C. H. Best). Department of Physiology, University of Toronto, Canada.

Increasing the calcium content of the environment of nerves of certain crustacea, frogs, fish and man lowered  $\lambda$ , the time constant of accommodation (hastened accommodation), and to a lesser degree lowered  $k$ , the time constant of excitation ( $0.693 k = \text{Chronaxie}$ ). An attempt has been made to correlate the quantity of ionized calcium present in the environment, as regulated by the percentage of citrate ion present with the absolute value of  $\lambda$  for frogs' sciatic nerves.

Accommodation became infinitely slow ( $\lambda$  very large), as shown by spontaneous activity of the nerve, when 16 per cent of the calcium in normal Ringer's solution existed in the ionized form. This represents a calcium ion concentration of 7.2 mgm. per cent. Increasing the unionized calcium (as calcium chloride), without increasing the ionized calcium above this low level, did not alter  $\lambda$ . Increasing the calcium ion concentration effected a reduction in  $\lambda$ . Using normal Ringer's solution with added sodium citrate, the calcium ion concentration at which  $\lambda$  became infinitely large was the same (within 10 per cent) for twelve preparations. No reasonable correlation between the absolute value of  $\lambda$  and higher calcium ion concentrations could be demonstrated, although, for any one preparation, the higher the calcium ion concentration the lower was the value of  $\lambda$  obtained. Using a solution containing no potassium, instead of the normal Ringer's solution previously employed, a very rough correlation between the absolute values of  $\lambda$  and ionized calcium present became evident.

Ionized calcium appears to be specific in its effect on  $\lambda$  in nerve, although there are other factors involved in fixing the absolute value of  $\lambda$ . One of these factors is the potassium content of the environment and another may well be normal variations in the calcium content of the calcium gel which constitutes the outer layer of the axon cylinder.

*Direct proof of the homeostatic regulation of the blood sugar by the liver.*

SAMUEL SOSKIN, HIRAM E. ESSEX, J. F. HERRICK and FRANK C. MANN.  
Metabolic Laboratory, Department of Physiology, Michael Reese Hospital, Chicago, and the Division of Experimental Medicine, The Mayo Foundation, Rochester, Minn.

A hepatic mechanism, which is chiefly responsible for the normal dextrose tolerance curve, has recently been described by Soskin and co-workers. Whenever the influx of exogenous sugar into the bloodstream raises the blood sugar level above the threshold of stimulation of this liver mechanism, there occurs a prompt diminution in the hepatic output of sugar to the blood. The exogenous sugar thus temporarily replaces the endogenous supply. Utilization and storage rapidly return the blood sugar towards its normal level, whereupon the liver again takes up its secretory function.

Direct observation of the operation of the above mechanism was made possible by the facilities of the Institute of Experimental Medicine, Mayo Clinic. Dogs which had completely recovered from a previous 2-stage ligation of the abdominal vena cava, just below the liver, were anesthetized with sodium amytal. A thermostromuhr unit was placed on the thoracic vena cava, just above the diaphragm, and another unit was placed on the portal vein. This enabled us to record the total outflow of blood from the liver, and to partition (by difference) the inflowing blood into its venous and arterial components. Samples of blood for determination of sugar content were drawn more or less simultaneously from the thoracic

vena cava, portal vein, and a carotid artery. We were thus able to calculate, in milligrams per minute, the total amount of sugar entering and leaving the liver at any given time. The intravenous administration of dextrose in these animals, whether in a single large injection or as a continuous small injection, yielded direct and unequivocal proof of the above described homeostatic liver mechanism.

The potential importance of this mechanism as a unifying conception in carbohydrate metabolism has been previously reported by Soskin and co-workers, in connection with observations on the dextrose tolerance curve under various experimental conditions. It was found that the various abnormalities following such diverse procedures as pancreatectomy or hypophysectomy on the one hand, or the administration of toxins on the other hand, could best be explained as being different disturbances of this same homeostatic liver mechanism.

*Blood pressure studies in normal and ovariectomized rats.* CLAIR R. SPEALMAN (by invitation) and ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

Preliminary records of arterial blood pressure obtained in the unanesthetized but immobilized rat by means of Hamilton's optical manometer showed a wide scattering for systolic and diastolic values. Heart rate studies (electrocardiographic) showed marked excitatory effects of gentle handling and of gentle immobilization, and pointed towards the following standard requirements which must be met if arterial blood pressure measurements on the rat are to have statistical significance; the animal must be quiet, warm, unexcited and free to move without alarming restraint. These requirements are apparently satisfied by placing the animal in a circular warmed cage, the floor of which is a turn-table, and using Bonsmann's photo-electric method for blood pressure in the tail. Pressure readings are taken only after the animal has become accustomed to its surroundings and shows indifference to them.

No suitable procedure has been developed to establish definitely the significance of these blood pressure values. However, application of this method to man gave agreement with systolic values simultaneously obtained by the auscultatory method.

Normal systolic pressure in the rat appears to be usually within the limits 100 to 130 mm. Hg.

A large series of determinations, in three groups of rats, normal females, spayed females and normal males, presented distribution curves which indicated significantly higher pressure levels in the normal male than in the normal female. The pressure levels in the group of spayed females were not significantly different from those of the normal females.

*Relative effects of iodoacetate and iodoacetamide on muscle respiration and glycolysis.* J. N. STANNARD (introduced by W. O. Fenn). Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

The threshold concentrations and relative rapidity of action of iodoacetate and iodoacetamide in inhibiting respiration and glycolysis of intact and minced frog muscle have been determined.

Lactic acid production is inhibited completely in two hours at  $1.0 \times 10^{-4}$  M iodoacetate and at  $2.7 \times 10^{-4}$  M iodoacetamide. No effect was observed

in 5 hours at lower concentrations. As the concentration is increased the iodoacetate inhibits much more rapidly, so that, at  $4 \times 10^{-4}$  M, iodoacetate is about four times as rapid as iodoacetamide. These results agree with those obtained by Smythe (J. Biol. Chem. **114**: 601, 1936) for inhibition of alcoholic fermentation in yeast. The physiological order of effectiveness of the two iodo compounds is in both cases the reverse of their order of effectiveness (rapidity) in combining with sulfhydryl groups. The problem is not one of penetration since the unionized iodoacetamide should penetrate the tissue more rapidly.

Oxygen consumption is inhibited by both compounds at approximately 10-fold higher concentrations. The thresholds are nearly identical, but as the concentration is increased it is the iodoacetamide which inhibits the more rapidly. At M/100, iodoacetate requires about  $1\frac{1}{2}$  hours, while iodoacetamide requires only 20 minutes for complete inhibition. The use of minced muscle does not change the results. Qualitatively, the two compounds behave towards muscle respiration as they do towards sulfhydryl groups; the reverse is true of lactic acid production.

Measurements of the respiratory quotient in concentrations of either poison which inhibit lactic acid production without affecting respiration indicated normal values ( $0.91 \pm 0.05$ ) for iodoacetate muscle, but a distinct rise to a value of 1.0 for iodoacetamide muscle. This difference may indicate differences in the action of the two iodo compounds in muscle.

*Further studies on a case of dysfunctioning salivary glands in man.* F. R. STEGGERDA and VICTOR BOUTEN (by invitation). Department of Physiology, University of Illinois, Urbana.

In an earlier report by one of us (F. R. S.) comparative studies were made of normal individuals and a college student devoid of active salivary glands (apparently congenital). These studies were concerned with the total fluid intake, the response to pilocarpine, and the threshold of taste to different solutions.

Although no salivary secretion was evident, frequently a slight amount of moisture was found on the inside of the cheeks and under the tongue of the subject. Therefore experiments were performed to test the presence of any enzyme activity in this secretion on starch paste. We also attempted to find whether there was any secretion of an iodide by way of the salivary or mucous glands in the mouth following the ingestion of 0.2 gram of potassium iodide by stomach.

The results of the first test indicate that there were no amylolytic enzymes in the subject's mouth; in the second test we found that there was no secretion of any iodide into the mouth after twenty-three minutes, whereas a positive test for iodide was recorded in the control subjects within thirteen to fourteen minutes following the ingestion of the material.

*The central differentiation of synchronized action potentials in the auditory nerves.* S. S. STEVENS and R. SOBEL (introduced by H. Davis). Department of Psychology, Harvard University, Boston, Mass.

The discovery that, from the eighth nerve, volleys of action potentials can be recorded which are synchronized with the pressure waves of the stimulating sound has led to a search for discriminatory processes based upon this synchronization. The effort to identify the sensation of pitch with the frequency of impulses in the auditory nerve fails on several counts:

1, the pitch of a tone can be changed without changing the frequency of the action potentials; 2, some people hear a different pitch in each ear for the same stimulating frequency (diplacusis), and 3, above 3000 cycles synchronization in the nerve breaks down, with no apparent effect on pitch perception.

On the other hand, the phenomenon of binaural beats, obtained when tones of slightly different frequency are led to each ear separately, appears to depend upon the central differentiation of the phase relations of synchronized nerve impulses. Due to its refractory phase, a single fiber in the auditory nerve can carry impulses in synchronism with the stimulus only for frequencies below about 800 cycles. At higher frequencies synchronization is obtained by the alternate activity of several fibers. Correlated with these facts is the observation that binaural beats occur only for frequencies below about 800 cycles. In the case of ten observers the frequency at which binaural beats ceased was between 750 and 800 cycles. Apparently central differentiation is possible when synchronization occurs in every active fiber, but not when it is due to the alternate activity of several fibers.

The simultaneous arrival centrally of impulses from the two ears produces an enhanced effect, as shown by the fact that in binaural beats the sensation is louder when the tones at the two ears are in phase and weaker when they are out of phase. There is also a slight tendency for the pitch to appear higher when the tones are in phase.

*Chronic effects of nearly total ablation of the cerebellum.* O. O. STOLAND, RICHARD H. GREER (by invitation) and ROZELLA BLOOD (by invitation). Department of Physiology, University of Kansas, Lawrence. (Motion picture.)

Moving pictures of a dog and photographs of its brain are presented which were taken ten years after very nearly complete extirpation of the cerebellum. The dog shows definite symptoms of ataxia, asynergia and asthenia. In walking the animal exhibits extensor hypertonia. These symptoms remained unchanged for a period of approximately ten years. The dog was otherwise in good health. A small remnant of the cerebellum the size of a bean was found at autopsy. The brain stem appeared intact.

*The  $O_2$  and  $CO_2$  tension in tissues following prolonged hyperventilation.*

R. T. STORMONT (by invitation) and M. H. SEEVERS. University of Wisconsin, Madison.

The tensions of  $O_2$  and  $CO_2$  in the skin and peritoneal cavity were determined by the gas depot method in twenty-four anesthetized or decerebrate dogs following moderate to maximal artificial hyperventilation of four to fifteen hours duration. The results from all experiments follow and are expressed in millimeter mercury tension at  $38^\circ C.$  and 740 mm. barometric pressure.

	SKIN		PERITONEAL CAVITY	
	$CO_2$	$O_2$	$CO_2$	$O_2$
Control (Averages).....	47	36	49	44
Hyperventilation:				
Average minimum.....	19	12	14	29
Individual minimum.....	9	1	7	13

It has been found impossible to reduce the  $\text{CO}_2$  in tissues below 7 mm. of mercury since greater ventilation results in lung rupture. The marked reduction in the  $\text{O}_2$  tension in the skin probably results from a compensatory peripheral vasoconstriction, as previously described by others. If the peritoneal  $\text{O}_2$  was reduced below 20 mm., the animals failed to survive. This condition did not obtain in these experiments except following lung rupture or hemorrhage.

*The application of the photoelectric colorimeter to the determination of carotene in blood serum, liver and feces.* GEORGE H. STUECK (by invitation) and ELAINE P. RALLI. Laboratories of the Department of Medicine, New York University College of Medicine, New York City. (Read by title.)

The errors in determining carotene occur chiefly in the reading of the extracted carotene. We have found the estimation of carotene by the photoelectric colorimeter a great improvement over the colorimetric and photometric methods.

For the extraction of carotene from blood serum either the White and Gordon or Clausen and McCord methods were used. From tissue, carotene was extracted with ether after digestion under nitrogen in an aqueous solution of KOH. This was washed and then dried with anhydrous sodium sulphate. The ether extract was reduced in volume and the residue taken up in cyclohexane or petroleic ether. Carotene was extracted from feces, after preliminary drying with alcohol, in a Soxhlet apparatus with ethyl alcohol and then ether. The ether alcohol fractions were combined, the solvents removed and the residue saponified. The unsaponifiable fraction was removed, the ether washed and dried and the sterols were then removed. The petroleum ether was made up to volume and read.

The solution of carotene obtained from blood, liver or feces was placed in the 10 cc. cuvettes used in the photoelectric colorimeter designed by Dr. B. Lange. A blue Jena glass filter no. BG12 with a maximum transmission at about 425 m $\mu$  was used as it was nearest to the maximum absorption band of carotene. Calibration curves were done with crystalline carotene (S.M.A.) in both petroleum ether (B.P. 40°-60°) and cyclohexane (Eastman Kodak White Label B.P. 81°). The latter was found superior due to the lessened loss by evaporation. The behavior of carotene in both solvents was the same as shown in table 1.

TABLE 1

SOLVENT	CAROTENE, MGM./100 ML.	NEG. LOG. TRANS- MISSION
Petroleic ether.....	0.10	0.10
Cyclohexane.....	0.10	0.10
Petroleic ether.....	0.40	0.33
Cyclohexane.....	0.40	0.33
Petroleic ether.....	0.65	0.40
Cyclohexane.....	0.65	0.40

*The non-centrifugal degeneration of severed peripheral nerve.* OSCAR SUGAR (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Chicago, Ill.

The left sciatic nerve of winter and spring frogs was severed near the cord, and the animals kept for 1 to 20 days at 18° to 20°C. When sacrificed (pithed), responses of both gastrocnemii to stimulation of various portions of their attached nerves were recorded; the nerves were then removed and their action potentials measured; finally, a histological examination of them was made.

The finding (Parker) that more distal stimulation of the nerve trunk, with weak shocks, evokes stronger muscle twitches than does proximal stimulation was confirmed—for control as well as sectioned nerve. It does not, therefore, signify a centrifugal degeneration but depends on the anatomical relations of the motor fibers to the whole nerve trunk. A given induced shock (Harvard inductorium, 3 volts in primary, secondary at 13 cm. and 5 to 10° off vertical) is more effective in exciting motor fibers to the gastrocnemius when fewer other fibers in the trunk shunt the current (stimulation distal to a branch is more effective than proximal) and when these motor fibers, which run in an eccentric bundle near the circumference of the trunk, are directly on the electrode (twisting the trunk on its longitudinal axis alters responses).

With such factors eliminated, it is found that the progressive increase of threshold with time, which occurs in the severed nerve, appears simultaneously throughout its length. Action potential studies are in harmony with this finding.

Histological examination of the trunk or of individual fibers teased out for 1 cm. or more also fails to support a centrifugal progression of degenerative changes.

The 1 or 2 cm. stretch next to the cut, and subject to traumatic degeneration, was excluded in all tests. A series run at 12 to 15° gave identical results but with a prolonged time scale.

*The effect of posture on human cardiac output.* H. MORROW SWEENEY (by invitation) and H. S. MAYERSON. Laboratory of Physiology, School of Medicine, Tulane University, New Orleans, La.

The cardiac output of five experienced subjects determined at intervals, for from four to six months, shows a decrease on quiet standing as contrasted to recumbency of from 5 to 26 per cent, with a mean difference of 15.8 per cent. Four observations on one subject in the sitting position show a decrease of 6.2 per cent.

Determinations (78 recumbent, 75 standing) with the Grollman method and the two-bag modification suggested by Gladstone agree closely for the recumbent position, but in the sitting and standing positions the Grollman method gives lower values than the Gladstone. Sampling at short intervals during the rebreathing in the recumbent position shows no evidence of recirculation over a period of 22 seconds, but in the sitting and standing positions the A-V O<sub>2</sub> differences increase progressively after about 9.5 seconds; at which time the rate of the acetylene diffusion from the lungs to the blood decreases abruptly, indicative of recirculation. The adequacy of the short Gladstone rebreathing procedure for attaining homogeneity of the gases in the lung-bag system prior to taking the first sample has been substantiated.

*A study of the effect of variations in blood flow through the liver on the flow of bile.* C. A. TANTURI (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago, Ill.

In view of the failure of Lundberg and Hillyard (1931) to obtain a change in bile flow following denervation of the liver in dogs with a biliary fistula, it was deemed advisable to repeat the work of Heidenhain and Munk on the effect of splanchnic section and stimulation on bile flow.

Section of the splanchnic nerves causes an increase in bile flow (7 of 8 dogs), confirming Heidenhain. This increase continues for at least 5 to 8 hours. Stimulation of the sectioned splanchnic peripherally generally causes a decrease in bile flow, though a slight temporary increase followed by a decrease, or only an increase may be observed which depends upon the strength of the stimulus and changes in systemic blood pressure. This occurs whether the spontaneous secretion or that excited by bile salt is being studied. Obstruction of the hepatic veins (left) causes a decrease in bile flow depending upon the degree of obstruction. Complete obstruction of the portal causes a complete or an almost complete suppression of bile flow. But, if the gastrointestinal tract and spleen are removed, some bile is formed for at least two hours. Obstruction of the hepatic artery (6 dogs) with or without the splanchnic or the hepatic nerves sectioned increases bile flow. Stimulation of the hepatic nerves generally decreases bile flow, although some variations occur, and section of the hepatic nerves increases bile flow. The hepatic nerves have been stimulated under various conditions and the results observed. Stimulation of the splanchnic nerve with the hepatic nerves sectioned usually causes a decrease in bile flow. Connecting the carotid or femoral artery to the splenic vein (portal) uniformly decreases bile flow. Hot or cold water irrigated through the colon usually decreases bile flow, the changes in systemic blood pressure being variable.

Bile flow appears to be modified more by the mechanical effects of vascular changes in the liver than by the effects of anoxemia.

*Is papain harmful?* SHIRO TASHIRO and L. H. SCHMIDT (by invitation). Biochemical Laboratory, University of Cincinnati, Cincinnati, O. (Read by title.)

Due to the increasing use of papain for softening meats the effect of the active papain on mammals was studied. It does not destroy the activity of human saliva, nor interfere with its speed of hydrolysis of starch. It is harmless to peptic digestion as the optimal condition of peptic digestion destroys papain, it does not interfere with the speed of peptic digestion, and as papain does not destroy pepsin. Neither does it retard lipolytic action of steapsin. A daily feeding of a large quantity of active papain to various mammals for two weeks produced no harmful effect in them as judged by appetite, growth, their general behavior and by complete absence of abnormality not only along the entire alimentary tracts, but also in various visceral and other vital organs of the fed animals. The maximum total amounts of active papain fed in two weeks were on the basis of 75 kgm. body weight of animals, 134.5 kgm. for mice, 4.5 kgm. for rat, 604.8 grams for guinea pig and 551.6 grams for dogs. These results indicate that so far as papain taken by mouth is concerned, it is harmless to mammals.

The papain is, however, harmful when applied to the skin (rabbits), or intraperitoneally injected in sufficient quantities (rabbits) as shown by others. The papain used was a commercial product, containing about  $\frac{1}{2}$  of the solid of papaya juice.

*The influence of morphine on colon motility in the dog.* R. D. TEMPLETON and E. A. GALAPEAUX (by invitation). Departments of Physiology, The University of Chicago and Loyola University School of Medicine, Chicago, Ill.

Prior to injecting morphine the colon motility of two trained, unanesthetized dogs was studied with two tandem balloon systems. One system of three balloons was inserted by way of a cecostomy and another system by way of the anus. Tracings of four hundred minutes duration were obtained on smoked paper once a week for a period of six weeks. The quantity of motility was calculated by considering a segment active when there was recorded a contraction or contractions lasting for one or more minutes. When there occurred a cessation in the activity of any segment lasting for two minutes or more that segment was considered quiet. Thus the calculated percentage of activity in the gut at any instance was dependent upon the activity recorded from six segments. For example, if during a given minute activity was being recorded from three segments while the remaining three were quiet the gut would be fifty per cent active. The first two hundred minutes of each tracing was considered as a control period to which the second two hundred minute period could be compared.

After obtaining a sufficient number of control experiments, one-quarter grain of morphine was injected daily and tracings obtained at intervals over a period of 124 days. When a tracing was being made morphine was injected at the close of the first two hundred minute period without interrupting the experiment. Since daily injections of morphine were given, two factors necessarily entered into the experiment: 1, the possible cumulative effect of morphine which, if measurable by colon motility, would be seen in the first two hundred minutes of the tracings; and 2, the immediate effect of morphine, which would be demonstrable only in the period immediately following the injection.

A summary reveals that one-quarter grain of morphine administered subcutaneously does not materially influence the motility of the colon on the following day, even after 124 daily injections. The immediate effect is an augmentation of activity throughout the two hundred minute period following the injection, and this effect remains practically unchanged after 124 daily administrations.

*Salt content of neural structures.* N. TUPIKOVA (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Chicago, Ill.

The importance of salts, in influencing the respiration and metabolism of neural tissue and its spontaneous activity and junctional transmission, is becoming ever more apparent. Information as to the salt content of various portions of the nervous system is consequently of interest in connection with their activity. Further, from the amounts of various ions, probable calculations can be made as to the fraction of the total volume of neural mass occupied by cells.

The average values for five dogs are given in the table. Under evipal anaesthesia, spinal fluid was rapidly drawn, then the animal bled fully and tissue samples dissected out as quickly as possible from the exsanguinated animal. Water was determined by drying to constant weight at 105°C., fats by refluxing the dry material in a thimble with petroleum ether. The residue was dry ashed in silica vessels and K measured by

Shohl and Bennett's iodoplatinate method, Na by Salit's microuranium-zinc method, Cl by Whitehorn's  $\text{AgNO}_3$  method.

The high K content of central structures, especially gray masses, is to be noted. The cerebellum, which shows the highest respiration, ascorbic acid content and frequency of electrical waves, also has the highest K content. Various portions of the C. N. S., white or gray, show essential homogeneity while peripheral nerve is sharply different.

	PER CENT FREE FAT IN FRESH TISSUE	PER CENT $\text{H}_2\text{O}$ IN		MM/KILO FAT-FREE FRESH TISSUE				
		Fresh tissue	Fat free fresh tissue	Cl-	Na**	K+	Phosphorus	
							Acid soluble	Total
Serum.....		92	92	123	159	4	1.4	4.9
C.S.F.....		99	92	134	149	3		
Muscle (gastroc.) right.....	2.8	75	77	17.5	28	93	40	51
Muscle (gastroc.) left.....	2.4	76	78	17.5	28	94	37	75.5
Nerve (sciatic) right.....	24.0	57	75	79	195	41	12	98
Nerve (sciatic) left.....	25.6	56	75	79	(171)†	(48)	12	107
Spinal cord.....	25	67	89	48	79	95	27	235
Medulla, pons.....	(20)	71	89	51	(75)	(77)	30	164
Midbrain.....	(15)	75	88	44	62	(89)	32	134
Thalamus.....	(14)	(75)	88	44	69	101	29	110
Caudate nucleus.....	(8)	77	84	39	64	86	36	114
Cerebellum.....	9	78	86	44	65	128	28	115
Cerebral white.....	19	70	86	43	58	108	26	150
Cerebral grey.....	7	80	86	47	70	103	28	82

\* Micro Na values average 16 per cent higher than by the macro method and are corrected.

† Bracketed values are averages of three or less observations.

*The response of the heart to exercises of graded intensity.* W. W. TUTTLE.  
Department of Physiology, College of Medicine, State University of  
Iowa, Iowa City.

The response of the heart to exercises of graded intensity was studied by means of the pulse-ratio method. The technique employed was as follows: Each subject was required to perform stool stepping at two different rates, for example, 18 and 40 steps, for one minute. A pulse ratio corresponding to each exercise was calculated by dividing the total pulse for two minutes immediately following the exercise by the resting pulse rate for one minute. Then the subject performed stool stepping at an intermediate rate, for example, 25 steps. The pulse ratio corresponding to this exercise was also calculated both experimentally and graphically. The pulse ratios for the first two exercises were plotted on the ordinate and the corresponding number of steps on the abscissa. By means of the straight line formula, the pulse ratio for the intermediate exercise was calculated and compared with that experimentally determined for the same exercise.

The group studied consisted of 117 individuals. The data showed that when the calculated and experimental pulse ratios for each individual in the group were compared, 104 of them coincided within 0.05 of a pulse ratio. The remaining 13 showed a difference of as much as 0.37.

Simultaneously with the pulse ratio test a cardio-vascular examination was made on each member of the group. This examination showed that in every case which failed to check, a non-compensated organic lesion was present. In order to check this point further 35 cases known to have a

non-compensated organic lesion were examined. In every case, the calculated and experimental ratios failed to coincide.

It was concluded from the experiment that the response of the normal heart to exercises of graded intensity is directly proportional to the intensity of the exercise and that the relationship is rectilinear. Furthermore, non-compensated hearts do not conform to this rule. The experiment not only proved the nature of the response of the normal heart to exercises of graded intensity but also provided a simple method for detecting non-compensated hearts.

*The effect of pregnancy on the heart weight/body weight ratio in the mammal.*

EDWARD J. VAN LIERE and CLARK K. SLEETH (by invitation). Department of Physiology, West Virginia University, Morgantown.

Guinea pigs selected for the purpose were used. They had not been used in any other work. They were fed an adequate diet. Seventy-two hours after parturition each animal was killed by a blow on the head. The body weight was determined and the heart removed. The great vessels were cut flush with the surface of the heart, and all four chambers were opened and washed free of blood. The excess moisture was removed by blotting the organ with filter paper. The heart was carefully weighed, and the ratio determined by dividing the heart weight in grams by the body weight in kilograms. Such data was obtained on twenty-four animals.

Another series of fifteen animals were killed at various periods of gestation. Their heart weight/body weight ratios were determined in a manner identical with that outlined above. The body weight was corrected for the uterine contents by reducing the uterus weight/body weight ratio to a predetermined normal and subtracting the excess uterine weight.

The heart weight/body weight ratio was found to be normal in both series of animals.

Since several determinations on cats corroborate the data found in the guinea pigs it would seem that the condition of pregnancy is not accompanied by cardiac hypertrophy in the mammal.

*Frequency and position of ovulation in the monkey ovary.* G. VAN WAGENEN and ARTHUR H. MORSE (by invitation). Yale University Medical School, New Haven, Conn. (Read by title.)

Ovulation or the absence of ovulation was determined for ninety-six cycles in eight macaques. For six animals the period of study continued more than a year. Laparotomies were performed usually on the fourteenth to twentieth days of successive cycles so that the surface of the ovaries could be examined. The quiescent ovary of the adult macaque is somewhat flattened dorso-ventrally with the straight anterior margin (hilus ovarii) attached to the broad ligament and the posterior curved margin is free. The dorsal and ventral surfaces of each ovary were considered separately and the position of the site of rupture was located in relation to the tubal or uterine end of the ovary and in the other direction in relation to hilum and free border. The place of rupture was plotted for the ovulation of the cycle in question and at the same time the position of stigmata and corpora of preceding cycles was checked. Ovulation was thought not to have occurred in only eight out of ninety-six observed cycles. The right ovary ovulated fifty-four times, the left, thirty-four.

The point of interest in these data lay in the fact that one ovary sometimes ovulated repeatedly for as many as five cycles. No significant difference in choice between dorsal and ventral surfaces appeared. There was a tendency for ovulation to take place toward the tubal end of the ovary (more than twice as often); and equally as often ovulation occurred toward the free margin rather than the hilus of the ovary.

*Urinary excretion of sodium pregnandiol glucuronide in the menstrual cycle.*

(An excretion product of progesterone.) ELEANOR M. VENNING (by invitation) and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

A study has been made of the excretion of sodium pregnandiol glucuronide in the urine throughout the human menstrual cycle. The gravimetric method described by one of us (Venning, in press) was used. The compound is considered to be an excretion product of progesterone. It is not excreted in the follicular phase but only in the luteal phase of the menstrual cycle. There is considerable variation both in the amount of the compound and the length of time over which it is excreted. Sufficient cases have not been studied to determine the limits of normal variation. Seven apparently healthy women were chosen for the investigation. The results are shown in the table, combined pregnandiol has been expressed in terms of pregnandiol, days are counted from the first day of the menstrual period, ovulation is estimated by the occurrence of intermenstrual pain or bleeding.

TABLE 1

CASE	AGE	OVULATION	COMPOUND APPEARED	COMPOUND DISAPPEARED	MENSTRU- ATION BEGAN	PREGNANDIOL
		day	day	day	day	mgm.
1	42		19	29	31	42.5
2	22	11	13	23	26	51.5
3	35 a	13	14	24	27	60.0
	b	12	14		29	?
	c	10	12	24	26	53.5
4	24		18	26	29	20.4
5	38		20	26	29	24.0
6	23 a		29	35	38	6.6
	b		23	26	29	3.0
7	45		12	19	20	15.0

The time of onset of the menstrual period in these cases bears a definite relation to the time of disappearance of the compound from the urine. It is suggested that the presence, the duration and perhaps the amount of the compound excreted may be used as an indication of the functional activity of the corpus luteum in the menstrual cycle.

*The relation of the reticulocytogenic urine principle to the hemopoietic or antipernicious anemia liver principle.* G. E. WAKERLIN. Department of Physiology and Pharmacology, University of Louisville School of Medicine, Louisville, Ky.

We and others have shown that normal human urine contains a principle which is reticulocytogenic for the pigeon, rat, and guinea pig, as is the hemopoietic or antipernicious anemia liver principle. The gross

chemical properties of the urinary and liver principles were found to be similar. A urine extract was prepared and demonstrated to be reticulocytogenic for the pigeon. The intramuscular administration of the urine extract to three suitable pernicious anemia patients was without effect, thus proving that the reticulocytogenic urinary substance is different from the hemopoietic liver principle. A parenteral kidney extract was likewise ineffective in two patients with pernicious anemia, although kidney by mouth is effective.

*Erythrocyte variations in normal men following insulin.* ORVILLE S. WALTERS, JAMES J. DELVECCHIO (by invitation) and ALRICK B. HERTZMAN. Departments of Physiology and Medicine, St. Louis University School of Medicine, St. Louis, Mo.

In this study the erythrocyte level has been employed as an indicator in an attempt to ascertain whether splenic contraction occurs in response to the adrenin secretion elicited by insulin hypoglycemia.

Ten fasting healthy men were used as subjects. Following a 30-minute period of rest in bed, a control blood sample was withdrawn, and ten units of insulin were given intravenously. Following this, blood samples were taken at intervals of 3, 15, 30, 45, 60 and 90 minutes. No carbohydrate was administered during the experimental period.

The erythrocyte count and volume of packed cells were determined, as well as the leucocyte count and "blood sugar" by the Shaffer-Hartmann method. Pulse and blood pressure were also followed.

Lowering of the "blood sugar" (uncorrected) to levels between 37 and 25 mgm. per cent occurred. Hypoglycemic symptoms of varying severity were noted in all subjects.

Two subjects showed an almost constant erythrocyte level throughout the experiment. Three showed fluctuations of the erythrocyte level without appreciable net increase. Five subjects showed a distinct rise in erythrocyte level, the maximum being reached at the 45 or 60-minute period, with a decline at 90 minutes.

Increase in sweat secretion occurred in nine of the ten subjects. The onset of the period of sensible perspiration was 30 to 35 minutes after the insulin and was most profuse between 40 and 50 minutes after.

Not only are the periods of erythrocyte rise and excessive sweat secretion coincident in time but the five men showing definite increase in erythrocyte level were those exhibiting the most profuse sweat secretion. These observations suggest dehydration as the cause of the increase in erythrocyte level.

*Bleeding tendency in chloroform poisoning.* E. D. WARNER (by invitation), K. M. BRINKHOUS (by invitation) and H. P. SMITH. Department of Pathology, State University of Iowa, Iowa City. (Transfer from Experimental Pathology.)

Large doses of chloroform administered to dogs causes a profound fall in plasma prothrombin, in addition to the well-known fall in fibrinogen. With smaller doses of chloroform, repeatedly administered, there is marked lowering of the prothrombin but not of the fibrinogen. Evidently the prothrombin level is more labile than that of the fibrinogen. With low prothrombin and normal fibrinogen levels the bleeding and clotting times are prolonged, and sometimes spontaneous hemorrhages occur.

*Relations of tissue pressure to venous pressure, capillary filtration and other factors.* HERBERT S. WELLS, DAVID G. MILLER, JR. (by invitation) and JOHN B. YOUMANS (by invitation). Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tenn.

We have found previously that the osmotic pressure of plasma proteins of venous blood from the foot increases 40 to 60 per cent on prolonged quiet standing, attaining final values of 45 to 55 cm. water. Since venous pressure in the leg averages about 90 cm. water (capillary pressure being therefore slightly above 90 cm. water) it is necessary to assume that tissue pressure must rise to at least 35 to 45 cm. water to stop filtration of fluid into the tissues of the leg. Since the leg volume, which rises rapidly at first, ceases to increase appreciably after 20 to 80 minutes at ordinary room temperatures one would expect to find tissue pressure elevated to values of 35 cm. water or higher after prolonged standing. Actually we find by direct measurement no significant elevation of subcutaneous or intracutaneous pressures (3 to 15 cm. covering the entire range of values). Intramuscular pressure, however, especially in muscles, as the anterior tibial and soleus, which are closely invested with a fascial sheath, does rise to values of the order of magnitude necessary to provide an effective anti-filtering force. Thus, intramuscular pressure in the anterior tibial is 5 to 10 cm. in the recumbent posture. With venous congestion the pressure rises at once in linear proportion to the elevation of venous pressure (showing that the muscle and its investing fascia serve to provide support for the blood vessels, as emphasized by Y. Henderson). In the erect posture intramuscular pressure rises at once to 20 to 35 cm. water, and a further progressive rise, paralleling the increase in leg volume, occurs, until values of 45 to 55 cm. are attained. Muscle tone also affects intramuscular pressure, but to a much less degree than might be expected.

It is concluded that filtration occurs chiefly into the muscles of the leg during venous congestion (however produced) and that it is in the muscles alone that a truly effective anti-filtering force develops.

*Time relations in renal excretion of threshold and no-threshold substances.*

H. L. WHITE and THOMAS FINDLEY, JR. (by invitation). Departments of Physiology and of Medicine, Washington University School of Medicine, St. Louis, Mo.

According to Verney's view that water diuresis results from a hypopitressinemia brought on by plasma dilution, the lag in increased water output behind plasma dilution represents the time required for plasma pitressin to fall. The alternative suggestion has been made that the lag represents the time required for the renal parenchyma to reach a new and higher state of hydration. If it can be shown that such a lag in increased output behind increased plasma concentration of the substance concerned exists for other threshold substances than water and does not exist for no-threshold substances, the finding would favor the second view and seem to make Verney's explanation unnecessary. It has been known for some time (Faber and Hansen, *Acta Med. Scand.* 58: 372, 1923) that such a lag exists for glucose. We have confirmed the water lag and have found a similar lag in chloride output behind increase in serum chloride on ingestion of sodium chloride in human subjects, changes in serum chloride being followed by a combination of conductivity and viscosity determinations capable of detecting a change of 1 mgm. per cent in sodium chloride. No

such lag exists, however, for the no-threshold substance, creatinine; an increase in serum creatinine produced by creatinine ingestion is immediately reflected in an increased creatinine output. It appears that tubular reabsorption of a threshold substance will continue unabated for some time after a plasma level has been established which will eventually bring about a diminution of reabsorption; in contrast, since tubular reabsorption of no-threshold substances does not take place at any plasma level, an increase in plasma concentration results in immediate increase in output.

*The effect of contraction of the intra-aural muscles on transmission of sound in the middle ear.* H. C. WIGGERS (introduced by H. Davis). Department of Physiology, Harvard Medical School, Boston, Mass.

The stapedius and tensor tympani muscles in guinea-pigs under dial-urethane anesthesia may contract spontaneously every few seconds. This allowed measurement of the effects of such contraction upon transmission of sound across the middle ear, inasmuch as with this anesthesia the muscles fail to respond reflexly to sound. Electrograms from the apex of the cochlea and from the round window during their activity revealed uniform patterns, from which the following deductions were drawn. 1. The deflections reflect the intralabyrinthine pressure changes due to ossicular movements and also the equilibration of interscalar pressure by movement of fluid through the helicotrema. 2. The stapedius responds prior to the tensor tympani, the latter contraction overpowering and outlasting the former. 3. These muscles perform both antagonistically and synergically.

The differences in intensity for each tone (250 to 2000 c.p.s.) required to elicit equal electrical responses during contraction and during rest, and the differences in intensity for lower tones (60 to 250 c.p.s.) necessary to produce threshold responses during and between contraction, yielded values which permitted construction of an "audiogram" which indicates the change in threshold caused by the contraction. The curve so constructed revealed that during contraction: 1, the loss in transmission increases linearly with the decrease of frequency (plotted logarithmically) from 1100 c.p.s. to 100 c.p.s., till at 100 it approximates 45 db; 2, from 1300 to 1800 the sensitivity increases above normal with the greatest increase in sensitivity at 1500 c.p.s.; and 3, the contraction does not affect the transmission of tones above 2000 c.p.s.

Apparently, therefore, the stapedius and tensor tympani may tune up the transmission apparatus in such a way that it becomes supersensitive to a few tones of medium frequency, but their predominant function is to effect a loss of sensitivity for a greater range of low tones, this loss being inversely proportional to the logarithm of the frequency. This implies a protective function against low tones of great amplitude, as was predictable from previous knowledge of mechanics of the middle ear. This may occur normally as the result of reflex contractions to sound stimuli which have previously been demonstrated.

*The gastric secretory curve before and after the Mann-Williamson operation and its bearing on the question of duodenal regurgitation.* CHARLES M. WILHELMJ and REX W. FINEGAN (by invitation). Departments of Physiology and Experimental Surgery, Creighton University, School of Medicine, Omaha, Neb.

In 90 per cent of 177 fractional gastric analyses on 26 normal dogs it

has been found that near the end of the gastric secretory curve there is an increase in the non-acid fluid entering the stomach which causes either a decrease in the acid concentration of the total secretions entering the stomach or prevents further rise. As this occurs, bile appears in the gastric contents. These changes are not seen in whole stomach pouches. We believe that these changes are due to the normal regurgitation of duodenal secretions into the stomach, although this opinion is not universally accepted.

Following the Mann-Williamson operation in which the duodenal secretions are drained into the terminal ileum, the acid concentration of the total secretions entering the stomach averages much higher than before operation. The non-acid fluid usually either remains unchanged or decreases near the end of the secretory curve and the acid concentration of the total secretions continues to rise or remains at a constant high level until emptying occurs. The average curve is thus similar to that found in whole stomach pouches and differs markedly from the average curve before operation. On rare occasions some non-acid fluid may regurgitate into the stomach from the jejunum which has been anastomosed to the stomach as was shown in a previous publication (Am. J. Physiol. **117**: 79, 1936).

These experiments show that regurgitation of duodenal secretion is a normal event and clearly define the rôle of regurgitation in the regulation of gastric acidity.

*Evidence against a pyloro-fundic reflex controlling acid secretion.* CHARLES M. WILHELMJ, REX W. FINEGAN (by invitation) and F. C. HILL (by invitation). Departments of Physiology and Experimental Surgery, Creighton University, School of Medicine, Omaha, Neb. (Read by title.)

The mechanism by which the pylorus augments the intragastric chemical phase of acid secretion has not been definitely established. Wilhelmj, O'Brien and Hill (Am. J. Physiol. **116**: 685, 1936) pointed out that there are certain facts which suggest that the effect may be due to a reflex from the pylorus to the fundus involving the intragastric nerve plexus. The following experiments were performed to test this hypothesis:

1. A dog was standardized by obtaining nine fractional analyses of the gastric acidity curve using a two per cent Liebig's extract test meal containing 15 mgm. of phenol red per liter.
2. Both vagus nerves were cut in the thorax. Two weeks after operation six fractional analyses were performed. There was an insignificant lowering of the acidity curve following vagotomy.
3. Four weeks after vagotomy the pylorus and antrum were completely severed from the fundus and immediately resutured, turning in about one-half inch of mucosa.
4. Three weeks after the second operation a series of fractional analyses were performed. There was no significant change in the gastric acidity curve.

These experiments do not support the hypothesis that the pylorus augments the secretion of acid during the intragastric chemical phase of acid secretion, by a pyloro-fundic reflex involving the intrinsic nerve plexus or the vagus nerves.

*Some factors governing initiation of respiration in birds and mammals.*

WILLIAM F. WINDLE (introduced by S. W. Ranson). Anatomical Laboratory, Northwestern University Medical School, Chicago, Ill.

Rhythmic respiratory movements appear normally in chicks about 3 days before hatching and in the late prenatal life of cats. In the chick, diminution in size of the allantoic vessels is a correlated phenomenon. Non-rhythmic chest and diaphragmatic contractions are present earlier. The integrated neck and trunk movements of very young chick and cat fetuses seem to become part of the respiratory pattern. Rhythmic respiratory movements can be induced experimentally before they occur normally.

Pinching allantoic or umbilical vessels in the last half of life in the egg or uterus was followed by 1, heightened irritability and quickening of fetal activity; 2, depressed irritability with disappearance of many exteroceptive reflexes, and 3, stimulation of deep rhythmic respiratory movements. These events occurred with less regularity when partial anoxemia was induced by administering nitrogen (chicks); impairment of heart action was associated with their occurrence.

Rhythmic respiratory movements were induced in all 14 to 19 day chicks in atmospheres containing an excess of oxygen and 1.6 per cent carbon-dioxide. Non-respiratory movements were depressed in 5 per cent carbon-dioxide, tonus of muscles was evident, and respiratory movements became more regular and deeper. In 10 per cent, deep, tonic, rhythmic respirations alone persisted. Responses to carbon-dioxide were more prompt than those following anoxemia.

Respirations were recorded by tambour connected with the air space of the egg. Both carbon-dioxide and oxygen-want apparently caused the lungs to open at 18 days and induced hatching at 20 days. Hyperpnea appeared in 1.6 to 3 per cent carbon-dioxide as well as in 14 per cent oxygen in about half the 20-day chicks which had begun to breathe air but whose allantois was still functioning. In others, the rate was unaffected or lowered slightly but the amplitude of breathing usually increased.

*On the mechanism of hypersensitivity produced by denervation.* H. G. WOLFF and McKEEN CATTELL. Departments of Pharmacology and Medicine, Cornell University Medical College and the New York Hospital, New York City. (Read by title.)

In 20 cats the nictitating membrane on one side was sensitized to epinephrine by section of the corresponding cervical sympathetic nerve (preganglionic) some days previously. The animal was anesthetized with "Dial" and the head firmly fixed in a holder. Both nictitating membranes were connected with isometric levers arranged for simultaneous photographic recording. Intravenous epinephrine injections (usually 1 cc. of 1:1,000,000) were made at frequent fixed intervals before and after stimulation of the postganglionic sympathetic fibers. Previous to nerve stimulation, the response of the membrane on the operated side averaged approximately 3 times that of the control. Stimulation of the sensitized side by a 30 to 90 seconds tetanizing stimulus caused a marked reduction in this difference and frequently the response on the two sides became equal. The sensitivity gradually returned over a period of about 20 minutes. Similar stimulation of the control side usually, but not always, resulted in an increase in the subsequent response to intravenous epineph-

rine. These results, coupled with the fact that preganglionic section is sufficient to bring about epinephrine hypersensitivity, suggest that in the absence of nerve impulses there is an accumulation of a substance (or state) in the effector cell which increases its excitability; conversely, this factor is reduced by nerve stimulation.

*Recordings of the separate contributions by the uterus and the abdomen to the expulsion of the child with simultaneous records of the maternal blood pressure in the systemic and placental arteries.* R. A. WOODBURY, W. F. HAMILTON, R. TORPIN (by invitation) and A. K. TEMPLES (by invitation). Departments of Physiology and Pharmacology and Obstetrics and Gynecology, University of Georgia School of Medicine, Augusta.

By means of differential manometers (see Hamilton and Woodbury, these abstracts) it is possible to subtract instrumentally the pressure in a gastric balloon from that in a balloon within the uterus and to record the resulting differential pressure curve. This will be referred to as the uterine component of the intra-uterine pressure. Between pains this pressure is 15 to 25 mm. Hg. During labor pains this pressure makes a smooth symmetrical curve reaching a height of 30 to 100 mm. Hg and lasting 90 seconds. Subtracting similarly the pressure in the cervical portion of the uterus from the pressure within the fundus of the uterus gives the actual expulsive force. Between pains this pressure amounts to less than 5 mm. Hg. But during the pains this increases 20 to 70 mm. Hg depending upon the stage of labor and upon the strength of the pain. "Bearing down" with the pains in the first stage does not increase the expulsive force though the intrauterine pressure may reach figures such as 170 mm. Hg. This shows the ineffectiveness of "bearing down" before the cervix is dilated.

Very interesting changes in the arterial pressure occur during strong uterine contractions. The brachial arterial pressure and the pulse pressure increase. With the differential manometer the uterine pressure was subtracted from the arterial pressure. This difference was recorded and gives an index to the effective placental pressure. During strong uterine contractions this net placental diastolic pressure may be below zero which means that maternal arterial blood may enter the uterus only during systole and flow back towards the aorta during diastole.

*Factors influencing micturition volume in the unanesthetized cat.* CLINTON N. WOOLSEY (by invitation) and CHANDLER McC. BROOKS. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

We have made an extensive series of observations to determine whether partial or complete removal of cerebral cortex influences frequency or volume of urination in the unanesthetized cat. The animals have been studied for long periods before and after operation by keeping each in a large metabolism cage equipped with apparatus for continuously recording volume and time of micturition. In order to maintain a fairly constant urine output the cats were placed on a standard diet which was the source of most of the water ingested. In the course of the investigation the influence of estrus, pregnancy and other factors was noted. The results were as follows:

1. Although the micturition volume of individual normal cats showed considerable variation from one urination to another, the average tended

to remain relatively constant. For most cats it fell between 50 and 70 cc., for a few around 25 cc., and for some others it was as much as 150 to 200 cc. These large urinations occurred in females.

2. Micturition volume diminished markedly during estrus or while the animal, female or male, was under the influence of Progynon B. In the female the decrease was correlated with vaginal smear and estrual behavior.

3. Tremendous increases in micturition volume have been observed postpartum.

4. Removal of periruciate (motor) cortex or ablation of the entire frontal pole, unilaterally or bilaterally, did not decrease urination volume, but often resulted in less marked variation in the volume of individual urinations.

5. Removal of entire neocortex, unilaterally or bilaterally, also diminished the variations in individual urination volume, but in some animals the average volume remained unaltered for months. In others there was a temporary increase in volume followed by a decrease of longer duration with a gradual stabilization at a value somewhat below that obtaining before operation. Further experiments must be done to determine whether such changes are due to loss of cortex or to injury of subcortical structures.

*An investigation, by electrical stimulation, into the function of motor and premotor cortex in the monkey (Macaca mulatta).* OSCAR A. M. WYSS<sup>1</sup> (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn. (Read by title.)

Stimulation of motor and premotor cortex in monkeys (*Macaca mulatta*) under very light ether anesthesia, with current pulses of optimum stimulating efficiency (utilization time at least 10 milli-seconds) reveals a characteristic difference between motor and premotor responses, whenever the rate of stimulation is kept as low as, say, two stimuli per second. Stimulation of motor foci in area 4 (Brodmann) gives a simple repetition of short twitches in the particular muscles of the contralateral side, each single twitch corresponding to each single stimulating shock; thus proving the cortico-spinal motor system to be of a rather simple type. If area 6 (Brodmann) is stimulated at the same rate a typical response appears after a latency, i.e., facilitation period of up to 10 or 15 seconds. This "pre-motor response" begins with a small twitch in some distal or proximal muscle, which becomes greater and greater, i.e., involves more and more muscles with each succeeding stimulus. No complete relaxation occurs between subsequent shocks. Each single contraction is sustained by itself. Each becomes superimposed on the preceding one and the whole motor response results in a progressive and purposeful movement (e.g., withdrawal of the leg, or adverse movement of the head). These premotor responses are no more elicitable after removal of area 4 or section between area 4 and area 6, nor have they been obtained from stimulation of area 4 after removal of area 6. They are apparently due to a combined activity of these two motor regions and are probably brought about by simultaneous action of the pyramidal and extrapyramidal projections.

It should be emphasized that in stimulation experiments on the central nervous system an accurate analysis of motor responses demands the

<sup>1</sup> Fellow of the Rockefeller Foundation.

lowest possible rate of stimuli. But each single current pulse should then be adapted to the time excitability of the neurones concerned, i.e., it should last long enough to be of maximum stimulating efficiency.

*Reflex production of inhibitory sympathin in unanesthetized dogs.* W. B. YOUMANS (by invitation) and W. J. MEEK. Department of Physiology, University of Wisconsin Medical School, Madison.

King (1924), Pearcy and Van Liere (1926), and Loew and Patterson (1935) have shown that gastro-intestinal inhibition may be produced by stimuli arising from the anus, rectum and colon. Belfield (1882) found that rectal stimulation produced a fall in blood pressure in normal animals but had no effect in splanchnicotomized ones.

We have found that rubbing the rectal mucosa with a small catheter sufficient to produce anal sphincter activity in unanesthetized dogs produces partial to complete inhibition of motility and decreased tonus in the stomach and in innervated or denervated jejunal fistulae. Coincident with the gastro-intestinal inhibition there is a decreased heart rate which returns to normal shortly after the cessation of rectal stimulation.

The intestinal and circulatory effects of rectal stimulation are not changed by vagotomy or by adrenalectomy, but are eliminated by splanchnicectomy and removal of the lumbar chains.

These facts indicate that during rectal stimulation inhibitory sympathin is produced reflexly in the gastro-intestinal tract in sufficient quantity to enter circulation and inhibit tissues not directly affected by the reflex.

*The action of hyoscine hydrobromide on developing Anura.* KARL YOUNGSTROM (introduced by Dr. O. O. Stoland). University of Kansas, Lawrence.

The action of hyoscine hydrobromide on Anuran larvae has been tested with the following results: About the time the animals begin to feed the growth rate begins to lag behind that of controls. Much less fecal matter is formed by the experimental animals than the controls, and during the course of one to three weeks, while the hyoscine treated animals may make feeding movements, they consume almost no food, and apparently starve in the presence of plenty. Behavior develops essentially normally, but with some retardation, until within a few days before death when the irritability to external stimulation decreases. Burr and Snively (1926), working with *Amblystoma* larvae treated with hyoscine, suggest that their results might be explained as due to the narcotic action on higher centers, possibly basal ganglia. While the primordium of the basal ganglia complex has been identified in the adult *amblystoma* (Herrick, 1927), such a complex is not mentioned by Coghill (1930) for the early swimming stage, hence the existence of such structures in the early swimming stage is at least doubtful. The failure of our hyoscine treated tadpoles to feed adequately and their consequent starvation suggests other possible explanations as for instance,

1. Retardation of prefacial and cerebral development which is occurring during the early swimming stage (Coghill, 1928).
2. Inhibition of visceral motor activity.
3. Interference with development of special senses.
4. Interference with some special phase of growth metabolism.

Available data tends to support the second and fourth possibilities.

*Comparative physiological development of some Anura.* KARL YOUNGSTROM (introduced by Dr. O. O. Stoland). University of Kansas, Lawrence. (Read by title.)

The developing behavior of several species of Anura has been followed from the premotile stage through metamorphosis. A chart of the data has been made for comparative purposes, (see exhibits). Some of the outstanding features are: 1, wide species variation in degree of development at time of hatching; 2, slight spontaneous quivering movement of the eyeball is the earliest evidence of eye muscle activity (compare earliest eye muscle movements in Toadfish, Tracy, 1926); later, spontaneous, bilateral, simultaneous, external divergence of the eyes develops, thus turning both eyes in the position of looking backward; this movement is followed by immediate return to the resting position. This type of movement was not observed in the species of toad studied. 3. Body reaction to rotation develops after the appearance of the spontaneous eye movements in all the frog tadpoles. 4. All forms have been free-swimming for a considerable time and give body reaction to rotation before legs appear. 5. Arms become motile 3 to 5 days before the legs (*Pseudacris triseriata*) but development of light touch response lags in the arms.

*The adrenals of rats following combined thyroidectomy and gonadectomy, considered in relation to pituitary histology.* ISOLDE T. ZECKWER. University of Pennsylvania Medical School, Philadelphia.

It is well known that pituitaries of castrated rats show basophilic "castration cells", with persistent acidophiles. Thyroidectomy causes disappearance of pituitary acidophiles and development of many basophilic "thyroidectomy cells" which the author previously showed are different histologically from "castration cells." Since when one adrenal is removed, compensatory hypertrophy of the opposite adrenal occurs only when the pituitary is present, it was thought that quantitative studies of compensatory adrenal hypertrophy after the pituitary histology was altered by thyroidectomy, gonadectomy, and combined thyroidectomy and gonadectomy, might indicate the type of pituitary cell responsible for adrenal growth.

Thyroidectomy, gonadectomy, or combination of the two operations was performed in rats five weeks old. Left adrenalectomy was performed in some at the same time; in others, four weeks later. All were killed when 16 weeks old.

In controls, with adrenals intact, thyroidectomy caused atrophy of adrenals in females, but not in males; gonadectomy caused hypertrophy in males, slight atrophy in females; combined thyroidectomy and gonadectomy caused hypertrophy in males but not in females. The factor of compensatory adrenal hypertrophy after thyroidectomy was taken as the weight of the right adrenal in thyroidectomized left adrenalectomized rats divided by the weight of the right adrenal of control thyroidectomized rats with both adrenals intact. Factors were similarly calculated for the other operations. The factor after thyroidectomy was increased (above the factor for unilaterally adrenalectomized rats with thyroids and gonads intact) in females, but not in males; after gonadectomy, was the same in males; after combined thyroidectomy and castration was not greatly altered if ratio to kidneys is considered.

Since the acidophiles disappear after thyroidectomy, yet compensatory

adrenal hypertrophy occurs, acidophiles can be excluded as responsible for adrenal growth.

Adrenotropic hormone presumably is not formed by "castration cells" or "thyroidectomy cells," since compensatory hypertrophy bears no proportion to their numbers.

*Potassium changes in experimental shock.* R. L. ZWEMER and J. SCUDDER (by invitation). Departments of Anatomy and Surgical Pathology, College of Physicians and Surgeons, Columbia University, New York City.

Fatal shock was produced experimentally in cats by recognized methods. Blood samples were obtained before and at intervals during the development of the syndrome, and their potassium content determined by an improved Truszkowski-Zwemer method. The K content of other body fluids was also determined in some cases. Disturbances in the usually constant blood K levels were found, with definite increases preceding death. High K levels were also found in the fluids obtained from closed body cavities. The absolute plasma, serum or whole blood K content does not appear to be as significant as the percentage and maintenance of the change which occurs. Rises of 50 to 100 per cent were not uncommon and the increases were always greater than errors of determination or of hemoconcentration. Animals brought into shock by extensive crushing of tissue, manipulation of viscera, acute pancreatitis, intestinal obstruction, or hemorrhage had blood potassium levels similar to animals with severe adrenal insufficiency, or shocked by intra-peritoneal injections of K salts (cf. Zwemer and Truszkowski, *Endocrinology* **21**: 40, 1937). Details of some experiments on intestinal obstruction have already been published (Scudder, Zwemer and Truszkowski, *Surgery* **1**: 74, 1937). The K rise after hemorrhage confirms the work of Kerr and of Thaler. Extensive work with histamine led Dale and his co-workers to suggest that it had a bearing on the problem of shock. Since the effect of excess potassium in extracellular body fluids is in many respects similar to that of histamine, and as injections of histamine are followed by increased blood potassium (Thaler), our findings are not inconsistent with those of Cannon, who holds that shock is due to a histamine-like substance.

*Some clinical effects accompanying changes in blood potassium experimentally produced.* R. L. ZWEMER. Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City. (Motion picture.)

The effects of acute potassium poisoning.

Experimental hyperpotassemia in a normal animal with subsequent recovery.

The potassium tolerance of an adrenalectomized animal as shown by injection of a very small amount of K.

Some results following injection of adrenal cortex extract into an animal with severe adrenal insufficiency.